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Fungicidal activity and chemical constitution

IX*. The activity of 6-*n*-alkyl-8-hydroxyquinolines

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(*Received* 5 *September* 1960)

SUMMARY

Maximum fungistatic activity in a series of 6-*n*-alkyloxines, tested against the mycelium of *Aspergillus niger*, was shown at a chain length of 6–7 carbon atoms. 6-*n*-Hexyloxine was more active than the corresponding 5-*n*-hexyl compound, the ED 50 value being approximately half.

In the presence of Cu^{2+}, maximum activity was found with oxine and 6-methyloxine; with some higher members of the series a rapid initial growth rate fell sharply after 2 days. This growth inhibition was shown to be due to the production of oxalic acid by the fungus, which resulted in the liberation of the free oxine.

INTRODUCTION

In an earlier paper in this series (Byrde, Clifford & Woodcock, 1958), the results of testing a number of 5-*n*-alkyloxines against the mycelium of *Aspergillus niger* van Tiegh were reported. When examined as unchelated molecules, i.e. in the presence of ethylenediamine tetra-acetic acid, disodium salt (Na EDTA), maximum activity was shown at a chain-length of 5–6 carbon atoms, whereas in the presence of metal ions such as Cu^{2+}, Fe^{2+} and Zn^{2+} maximum activity was found with oxine and 5-methyloxine. These findings were discussed in terms of optimum lipoid solubility and the critical effect of pH on the fungistatic activity of the unchelated oxine homologues was emphasized. Certain anomalies observed in the mycelial growth tests when metals were present were also noted and possible explanations mentioned.

The present work with the corresponding series of 6-*n*-alkyloxines was planned to provide further evidence for the role played by chemical structure, through chelation and lipoid solubility, in the fungistatic activity of 8-hydroxyquinoline.

MATERIALS AND METHODS

Synthesis of the eight 6-*n*-alkyl-8-hydroxyquinolines is described elsewhere (Clifford, Davis & Woodcock, 1960).

The methods used for assaying fungistatic activity against mycelium and for spore germination tests were as previously described (Byrde *et al.* 1958).

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EXPERIMENTAL RESULTS

Fungistatic activity against mycelium. Effect of n-*alkyl side-chain on activity of unchelated molecules*

The mean mycelial growth of *A. niger* in the presence of oxine and eight 6-*n*-alkyl homologues at concentrations of 10^{-4} and 5×10^{-5}M is shown in Table 1; Na EDTA was present at 10^{-4}M throughout. Least growth was recorded at a chain length of 5–7 carbon atoms and these compounds were further tested at a range of concentrations with the results shown in Table 2. Based on ED 50 values the fungistatic activity of the pentyl, hexyl and heptyl homologues increased with increasing molecular weights.

Table 1. *Mean mycelial growth (mm./day) of* Aspergillus niger *in the presence of a series of* 6-n-*alkyl-8-hydroxyquinolines and* Na EDTA (10^{-4}M)

No of carbon atoms in alkyl side-chain	Fungicide concn. (M)	
	10^{-4}	5×10^{-5}
0	8·82	Not tested
1	8·32	Not tested
2	11·32	Not tested
3	8·04	7·50
4	0·00	7·94
5	0·00	0·00
6	0·00	0·56
7	0·00	0·00
8	2·19	3·32
Control	9·37	
Least significant difference ($P = 0{\cdot}05$)	1·56	

Table 2. *Dosage-response data for three* 6-n-*alkyl-8-hydroxyquinolines against mycelial growth of* Aspergillus niger

Compound	Equation of probit regression line	10^6 ED 50 (molar)*	Slope of probit regression line
6-*n*-Pentyl-8-hydroxy-quinoline	$Y = 13{\cdot}70 - 6{\cdot}31x$	23·97 (17·17–31·04)	$-6{\cdot}31 \pm 1{\cdot}04$
6-*n*-Hexyl-8-hydroxy-quinoline	$Y = 11{\cdot}90 - 5{\cdot}86x$	15·03 (10·61–21·41)	$-5{\cdot}86 \pm 0{\cdot}71$
6-*n*-Heptyl-8-hydroxy-quinoline	$Y = 8{\cdot}63 - 3{\cdot}57x$	10·39 (6·61–15·72)	$-3{\cdot}57 \pm 0{\cdot}68$

* 5 % fiducial limits are shown in parentheses.

Comparison of fungistatic activity of 5-n-*alkyl- and* 6-n-*alkyloxines*

In view of the earlier results with the 5-*n*-alkyloxines in which the maximum fungistatic activity occurred at a chain length of 5–6 carbon atoms, the *n*-hexyl homologues of both series were compared together with 6-*n*-heptyloxine which was of similar activity. The results, given in Table 3, show that the ED 50 value for 5-*n*-hexyloxine was 1·98 times greater than that for the corresponding 6-*n*-hexyl compound, which in this test showed comparable activity to 6-*n*-heptyloxine.

Table 3. *Dosage response for* 5-n-*hexyl*-8-*hydroxyquinoline,* 6-n-*hexyl*-8-*hydroxyquinoline and* 6-n-*heptyl*-8-*hydroxyquinoline*

Compound	Equation of probit regression line	10^6ED 50 (molar)*	Slope of probit regression line
5-*n*-Hexyl-8-hydroxy-quinoline	$Y = 13{\cdot}33 - 5{\cdot}63x$	30·05 (21·14–40·61)	$-5{\cdot}63 \pm 1{\cdot}41$
6-*n*-Hexyl-8-hydroxy-quinoline	$Y = 8{\cdot}94 - 3{\cdot}34x$	15·16 (10·24–20·65)	$-3{\cdot}34 \pm 0{\cdot}62$
6-*n*-Heptyl-8-hydroxy-quinoline	$Y = 13{\cdot}51 - 7{\cdot}16x$	15·46 (12·61–19·01)	$-7{\cdot}16 \pm 1{\cdot}34$

* 5 % fiducial limits are shown in parentheses.

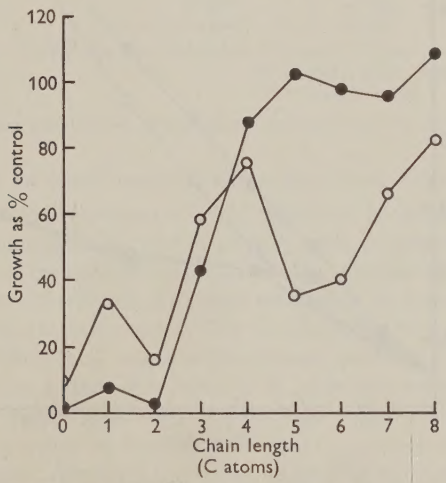

Fig. 1. Effect of chain length of 6-*n*-alkyl-8-hydroxyquinolines (10^{-4}M) on mycelial growth of *A. niger* in the presence of Cu^{2+} (10^{-3}M), expressed as percentage of growth of cultures with Cu^{2+} only. ●, 0–2 day growth; ○, 2–4 day growth.

The effect of added metals

Fig. 1 shows the effect on the growth of *A. niger* of the 6-*n*-alkyloxines in the presence of added Cu^{2+}. The 0–2 day growth showed a significant reduction only for the lower homologues, whereas the corresponding values for 2–4 days showed a marked depression at a chain length of 5–6 carbon atoms. This effect is similar to that noted earlier for the 5-*n*-alkyloxines (Byrde *et al.* 1958) although in that case the effect was not apparent until 4–6 days after inoculation. A comparison of growth rates between selected members of the two series was therefore made with the results shown in Table 4. This shows that, whereas growth is significantly retarded at 2–4 days with the 6-*n*-alkyl series, the effect is delayed until 4–7 days with the 5-*n*-alkyl homologues.

Fig. 2 shows the results of a more detailed experiment on the effects of Cu^{2+} and Fe^{2+} on the growth of *A. niger* in the presence and absence of 6-*n*-hexyloxine; each point is the mean of twelve observations. Statistical analysis of the data summarized in Table 5 shows highly significant decreases in the growth rates with time of cultures in the presence of either metal and the oxine homologue. In such cultures where growth became inhibited, the fungus colony had a characteristic 'hard' edge and was surrounded by a ring of almost colour-free agar in which the pH was found to

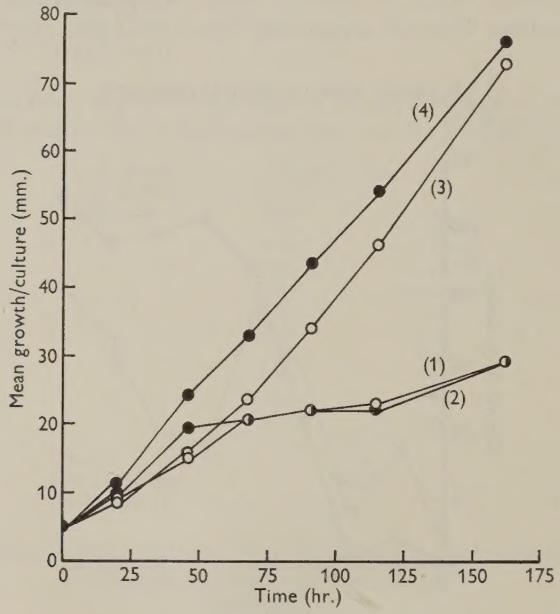

Fig. 2. Growth curves of *A. niger* in the presence of Cu^{2+} and 6-*n*-hexyloxine (1), Fe^{2+} and 6-*n*-hexyloxine (2), Cu^{2+} alone (3) and Fe^{2+} alone (4).

Table 4. *Mean mycelial growth of* Aspergillus niger *in the presence of* Cu^{2+} (10^{-3}M) *and three homologues from the* 5-n-*alkyl- and* 6-n-*alkyl*-8-*hydroxyquinoline series* (10^{-4}M)

Series	No. carbon atoms in side-chain	Mean growth (mm./day) 0–2 days	2–4 days	4–7 days
5-Alkyl	5	7·13	9·63	7·50**
	6	6·50	11·63	8·29**
	7	7·06	10·50	8·92*
6-Alkyl	5	6·75	5·38**	4·17***
	6	6·63	4·25**	1·54***
	7	6·25	6·50*	0·46***
Copper only	—	6·44	10·88	12·71
Control (no copper)	—	8·87*	11·44	12·04

Asterisks denote values significantly different from that for the 'copper only' treatment.

have fallen from 4·3 (original medium) to 3·1. This suggests that the metal/6-*n*-hexyloxine complex was being broken down by some fungal metabolite and the 6-*n*-hexyloxine thus liberated was inhibiting fungal growth. It was later found that these effects could be simulated by the addition of the filtrate from 4-day-old cultures of *A. niger* grown on the liquid medium previously described (Byrde, Harris & Woodcock, 1956).

Table 5. *Mean mycelial growth (mm.) of* Aspergillus niger *in the presence of metals and* 6-n-*hexyl-8-hydroxyquinoline*

	2-day period				
Treatment	1–3		3–5		5–7
6-*n*-Hexyloxine + Cu^{2+}	10·125	***	2·375	*	5·750
6-*n*-Hexyloxine + Fe^{2+}	11·750	***	2·375	*	5·625
Cu^{2+} only	14·875		22·625	**	26·625
Fe^{2+} only	20·750		21·375		22·250
Control	19·750	*	23·000		24·250

Least significant difference ($P = 0{\cdot}05$): 2·592
($P = 0{\cdot}01$): 3·470
($P = 0{\cdot}001$): 4·559

Asterisks denote levels of significance between the time periods.

Isolation of the metal complex splitting factor from culture filtrates

6-*n*-Hexyloxine (10^{-2}M in ethanol, 0·1 ml.) added to McIlvain citrate-phosphate buffer, pH 3, containing $CuSO_4$ (10^{-4}M, 10 ml.), gave a yellowish-green coloration due to the formation of copper 6-*n*-hexyloxinate. The addition of an equal volume of the filtrate from 4-day-old cultures of *A. niger* resulted in an immediate loss of colour due to breakdown of the metal complex. This decolorization was used to demonstrate complex-breaking activity at all stages in the isolation procedure.

The filtrate (1 l.) from 4-day-old cultures of *A. niger* was concentrated to 100 ml. by rotary film evaporation *in vacuo* at 37° C. and then electro-dialysed by the method of Wood (1956) using a flow of demineralized water, a voltage of 160 V. d.c. and a current of 0·05–0·2 amp. After 4–5 hr. it was found that most of the 'activity' was located in the anode compartment. This solution was concentrated *in vacuo* and finally evaporated to dryness in a desiccator. The residual solid was extracted with ether and crystallized from ether–light petroleum (b.p. 40–60° C.). On heating, this acidic product lost water at 101–102° C. and finally melted with decomposition at 186° C. (Found: C, 19·6; H, 4·7. Calc. for $C_2H_2O_4.2H_2O$; C, 19·0; H, 4·8%.) The m.p. was undepressed by admixture with oxalic acid.

The amount of oxalic acid present in 4-day-old culture filtrates was found to be 4·4 g./l. by precipitation as calcium salt from a strongly acetic acid solution, re-solution of the precipitate in 2N H_2SO_4 and titration against N/10 $KMnO_4$.

To demonstrate the complex-breaking activity of oxalic acid, 6-*n*-hexyloxine (10^{-4}M in chloroform) (A) was treated with an excess of aq. $CuSO_4$. The colourless chloroform layer which became deep yellow (B) was then shaken with a saturated aqueous solution of oxalic acid when it was decolorized (C). Solutions A, B and C were examined in a Unicam SP500 spectrophotometer, the absorption curves being shown in Fig. 3. Curve B shows clearly that the absorption band for 6-*n*-hexyloxine

(294–336 mμ) has been replaced by that of copper 6-*n*-hexyloxinate at 388–410 mμ. Addition of excess oxalic acid restores the band to its original position.

Chromatographic examination of the culture filtrates showed the presence of several keto-acids, but treatment with 2,4-dinitrophenylhydrazine (Block, Durrum & Zweig, 1958) failed to reduce complex-breaking activity.

Table 6. *Effect of* 6-n-*hexyloxine* (10^{-4}M) *on mycelial growth of* Aspergillus niger *in presence and absence of oxalic acid.* Cu^{2+} *present at* 10^{-3}M *throughout*

	Mean mycelial growth (mm.)					
	1–2 days			2–3 days		
Oxalic acid (%)	+6-*n*-Hexyloxine		Control	+6-*n*-Hexyloxine		Control
0·125	1·875 *** ***	***	12·000	2·625	***	12·375
0·0625	7·250	***	12·000	1·625	***	13·250
0·03125	9·625		11·875	2·500	***	11·625
0	8·375		10·125	3·000	***	12·625

*** Difference significant ($P = 0{\cdot}001$).

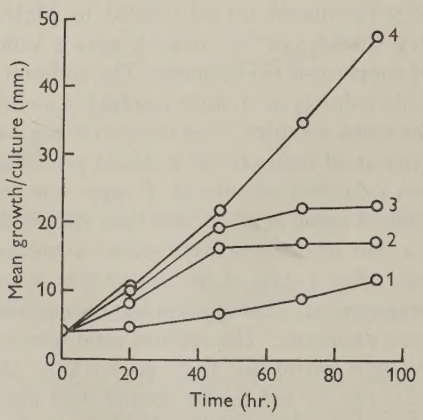

Fig. 3. Growth curves of *A. niger* in the presence of Cu^{2+} and 6-*n*-hexyloxine with added oxalic acid, as follows: (1) 0·125 %, (2) 0·0625 %, (3) Nil. Growth with Cu^{2+} only, shown by (4), was not reduced significantly by oxalic acid at any level (see Table 6).

Effect of oxalic acid secretion on growth curves

To demonstrate the biological effect of oxalic acid on the growth of *A. niger*, a sterile solution of oxalic acid was incorporated in the agar medium at the time of pouring and before the addition of the copper and 6-*n*-hexyloxine. Table 6 and Fig. 3, summarizing the results obtained, show that whilst oxalic acid in the presence of copper alone had little effect, its addition at 0·0625 or 0·125 % resulted in a marked reduction of the growth rate at an early stage when 6-*n*-hexyloxine was present.

DISCUSSION

The results described for the 6-*n*-alkyloxines follow the general pattern found previously for the corresponding 5-*n*-alkyl series. Maximum fungistatic activity, however, was associated with a slightly greater chain length (6–7 carbon atoms) and these

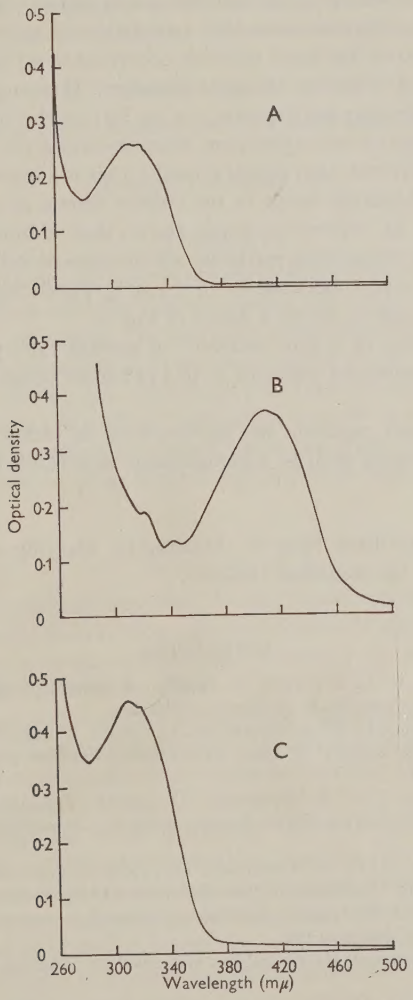

Fig. 4. Absorption curves for solutions A, B and C (see text).

homologues were approximately twice as active as 5-*n*-hexyloxine. The reason for this greater activity is not clear in the light of present evidence though differences in molecular shape, solubility or electronic configuration may be involved. The very

low solubility in water or aqueous ethanol unfortunately precluded determination of pK values of the higher members.

In the previous paper it was noted that the growth of *A. niger* in the presence of 5-*n*-pentyloxine and Cu^{2+} was unexpectedly retarded after a period of 4 days. It was shown that this was not a direct effect of a change of pH due to acid production by the fungus and it was tentatively suggested that some fungal detoxication mechanism was operative. In the present work, the retardation of growth in the presence of Cu^{2+} and 6-*n*-hexyloxine was more marked, occurring at an earlier stage of mycelial growth and amounting to almost complete cessation. It is suggested that oxalic acid, produced in substantial amount (approx. 5×10^{-2}M) by the fungus, sequestered the copper from the copper 6-*n*-hexyloxinate, thus liberating the fungistatic 6-*n*-hexyloxine which then interfered with fungal growth. This is supported by the identification of the complex-breaking factor in the culture filtrate as oxalic acid and by the absorption data (Fig. 4). Moreover, it was shown that addition of oxalic acid to the medium at the time of inoculation markedly affected growth rate; inhibition of growth was immediate at an acid concentration of 0·125%, producing a growth curve very similar to the later stages of curves 1 and 2 of Fig. 2.

These show evidence of a slow recovery of growth rate, possibly due to either degradation of the unchelated molecule or to a pH effect caused by further oxalic acid production.

These results do not preclude the participation of other competing chelating agents although keto-acids such as 2-ketogluconic acid (Duff & Webley, 1959) were clearly not involved.

The authors wish to thank Miss M. Mealing for carrying out the bio-assays and Mr G. M. Clarke for the statistical analyses.

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Investigations on fungitoxic derivatives of salicylaldehyde

II. Chlorinated and brominated derivatives of salicylideneaniline and related compounds as eradicants of cucumber powdery mildew (*Erysiphe cichoracearum* DC.)

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(*Received* 19 *August* 1960)

SUMMARY

A number of halogenated derivatives of salicylideneaniline were synthesized, several of which showed high eradicative activity against cucumber powdery mildew when sprayed on to young pot plants. The two most promising compounds were 3-bromo- and 3-chloro-salicylideneaniline. These were subjected to commercial-scale trial, but, although more effective (at 0·08–0·10%) than standard 2:4-dinitro-6-(1-methylheptyl)phenyl crotonate ('Karathane') sprays in controlling severe mildew infection, they showed phytotoxic effects on mature cucumber plants.

Their toxicity to certain other fungi is outlined in the appendix.

INTRODUCTION

The formation of salicylideneaniline, by the condensation of salicylaldehyde and aniline, was reported by Schiff (1869), who also observed that this compound combined with certain metals; a property (chelation) possessed by certain highly fungitoxic compounds, such as 8-hydroxyquinoline.

The toxicity of salicylideneaniline to two fungi infecting human skin was observed by Okazaki, Kawaguchi, Matsui & Kitamura (1952), and the marked *in vitro* fungitoxic activity of eleven derivatives of 5-chlorosalicylideneaniline has since been determined by Reisner & Borick (1955).

Since salicylideneaniline is the 2-hydroxy derivative of benzylideneaniline, and since certain chlorinated benzylideneanilines have been found, in a previous study (Read, Smith & Hughes, 1953), to have a high toxicity towards eggs of the glasshouse red spider mite (*Tetranychus urticae*), the fungicidal and acaricidal activities of some chlorinated derivatives of salicylideneaniline were investigated with the ultimate aim of finding a material able to provide a joint control of cucumber mildew and mites. The present investigation, necessitating the preparation of a number of derivatives of salicylideneaniline (which in preliminary tests showed low acaricidal values), was limited to the search for a more satisfactory fungicide for the control of powdery mildews.

Eradicative, in addition to protective, activity is important in the evaluation of

chemicals for the control of cucumber powdery mildew, since growers are frequently able to limit attacks by careful regulation of cultural conditions, and a routine programme of spraying to prevent infection is not usually adopted. Fungicides having an eradicative action in addition to a protective action are also desirable because the very rapid growth of the plants necessitates frequent applications of a protective fungicide, and the *efficient* application of sprays to glasshouse-grown cucumbers is laborious and expensive. Furthermore, 'Karathene' and copper oxychloride/petroleum, the fungicides at present available for the control of this disease, are often mildly phytotoxic.

EXPERIMENTAL

Chemicals

The experimental compounds were prepared by condensation between salicylaldehyde, or the appropriate derivative of salicylaldehyde, and aniline, or the appropriate monochlorinated aniline, in warm ethanol. The required compound was precipitated by cooling, assisted by the addition of water if necessary, filtered off and recrystallized to constant melting point.

Micro-analyses were carried out by Drs G. Weiler and F. B. Strauss, Oxford.

Salicylideneaniline. Crystallized from light petrol in small yellow needles, m.p. 51° C. (Found: C, 79·45; H, 5·5. Calc. for $C_{13}H_{11}NO$: C, 79·65; H, 5·6 %.) The literature m.p. is 51° C.

Salicylidene-4-chloroaniline. Crystallized from ethanol in long orange-yellow tabular needles, m.p. 103·5° C. (Found: Cl, 15·4. Calc. for $C_{13}H_{10}NOCl$: Cl, 15·3 %.) Senier & Shepheard (1909) give m.p. 102–103° C.

3-*Chlorosalicylideneaniline.* 3-Chlorosalicylaldehyde (m.p. 56° C.; Davies & Rubenstein, 1923, give m.p. 55° C.) was prepared by subjecting *o*-chlorophenol to the Reimer–Tiemann reaction, and was condensed with aniline in warm ethanol. The product crystallized from ethanol in short, pale orange needles, m.p. 59·5–60·0° C. (Found: C, 68·0; H, 4·25; N, 6·4; Cl, 15·1. Calc. for $C_{13}H_{10}NOCl$: C, 67·35; H, 4·35; N, 6·05; Cl, 15·3 %.) Hydrolysis of this material by boiling 10 % hydrochloric acid gave 3-chlorosalicylaldehyde (mixed m.p. determination).

3-*Chlorosalicylidene-2-chloroaniline.* Crystallized from 80 % acetic acid in golden-yellow needles, m.p. 142·0–142·5° C. (Found: C, 58·8; H, 3·1. Calc. for $C_{13}H_9NOCl_2$: C, 58·6; H, 3·4 %.)

3-*Chlorosalicylidene-3-chloroaniline.* Crystallized from 80 % ethanol in short deep-yellow prisms, m.p. 104° C. (Found: C, 58·6; H, 3·5. Calc. for $C_{13}H_9NOCl_2$: C, 58·6; H, 3·4 %.)

3-*Chlorosalicylidene-4-chloroaniline.* Crystallized from ethanol in orange prisms, m.p. 108–109° C. (Found: C, 58·0; H, 3·6. Calc. for $C_{13}H_9NOCl_2$: C, 58·6; H, 3·4 %.)

4-*Chlorosalicylideneaniline.* 4-Chlorosalicylaldehyde (m.p. 53° C.) was prepared from *m*-chlorophenol (Hodgson & Jenkinson, 1927, who give m.p. 52·5° C.), and was condensed with aniline in warm ethanol. The product crystallized from 80 % ethanol in minute deep-yellow needles, m.p. 69·5° C. (Found: C, 67·5; H, 4·2. Calc. for $C_{13}H_{10}NOCl$: C, 67·35; H, 4·35 %.)

5-*Chlorosalicylideneaniline.* 5-Chlorosalicylaldehyde (m.p. 101–102° C.) was prepared by the action of chlorine on hot salicylaldehyde (Biltz & Stepf, 1904, who give m.p. 99·5° C.). (Found: Cl, 22·2. Calc. for $C_7H_5O_2Cl$: Cl, 22·6 %.)

5-Chlorosalicylideneaniline crystallized from ethanol in long, flat orange needles, m.p. 109–109·5° C. (Found: C, 67·7; H, 4·7. Calc. for $C_{13}H_{10}NOCl$: C, 67·35; H, 4·35 %) and from benzene/ligroin, m.p. 109·5° C. Reisner & Borick (1955) give m.p. 111–112° C.

5-*Chlorosalicylidene-4-chloroaniline.* Crystallized from glacial acetic acid and benzene/ligroin in deep-yellow platelets, m.p. 147–148° C. (Found: C, 59·1; H, 3·6. Calc. for $C_{13}H_9NOCl_2$: C, 58·6; H, 3·4 %.) Reisner & Borick (1955) give m.p. 149·5–150·5° C.

3:5-*Dichlorosalicylideneaniline.* 3:5-Dichlorosalicylaldehyde (m.p. 95° C.) was prepared by

the action of chlorine on a hot solution of salicylaldehyde in glacial acetic acid (Claisen & Tietze, 1926, who give m.p. 95° C.). (Found: Cl, 37·2. Calc. for $C_7H_4O_2Cl_2$: Cl, 37·15 %.)

3:5-Dichlorosalicylideneaniline crystallized from ethanol in orange microcrystals, m.p. 102–103° C. (Found: C, 59·0; H, 3·65; N, 5·5. Calc. for $C_{13}H_9NOCl_2$: C, 58·6; H, 3·4; N, 5·25 %.)

3:5-*Dichlorosalicylidene-4-chloroaniline.* Crystallized from 90 % acetic acid in red-orange needles, m.p. 139–140° C. (Found: C, 51·7; H, 2·7. Calc. for $C_{13}H_8NOCl_3$: C, 51·9; H, 2·7 %.)

3-*Chloro-5-methylsalicylideneaniline.* 3-Chloro-5-methylsalicylaldehyde was prepared from 3-chloro-*p*-cresol by the Reimer–Tiemann reaction. Recrystallization from 90 % methanol gave pale cream needles, m.p. 63·5–64·5° C. (Found: Cl, 20·6. Calc. for $C_8H_7O_2Cl$: Cl, 20·8 %.)

3-Chloro-5-methylsalicylideneaniline crystallized from 90 % ethanol in small, tangerine-coloured needles, m.p. 51·0–52·5° C. (Found: C, 68·5; H, 4·9; N, 5·6. Calc. for $C_{14}H_{12}NOCl$: C, 68·4; H, 4·9; N, 5·7 %.)

3-*Bromosalicylideneaniline.* 3-Bromosalicylaldehyde was prepared from *o*-bromophenol by the Reimer–Tiemann reaction, and crystallized from water in small white needles, m.p. 54·0–54·5° C. (Müller, 1909, gives m.p. 49° C.) (Found: Br, 39·5. Calc. for $C_7H_5O_2Br$: Br, 39·7 %.)

3-Bromosalicylideneaniline crystallized from 80 % ethanol in short orange needles, m.p. 70·5° C. (Found: C, 57·0; H, 3·8; N, 5·05. Calc. for $C_{13}H_{10}NOBr$: C, 56·5; H, 3·65; N, 5·05 %.)

3-*Bromo-5-chlorosalicylideneaniline.* 3-Bromo-5-chlorosalicylaldehyde was prepared by the gradual addition of bromine (30 g.), in glacial acetic acid (20 ml.), to a solution of 5-chlorosalicylaldehyde (29 g.) in glacial acetic acid (90 ml.), at *c.* 60° C. The reaction mixture was refluxed for 2 hr. and allowed to cool after the addition of water (35 ml.). The solid product crystallized from 90 % ethanol in cream-coloured needles, m.p. 86° C. (Found: C, 36·5; H, 1·9. Calc. for $C_7H_4O_2ClBr$: C, 35·7; H, 1·7 %.)

3-Bromo-5-chlorosalicylideneaniline crystallized from ethanol in deep-orange microcrystals, m.p. 86–87° C. (Found: N, 4·7; Cl and Br, 37·8. Calc. for $C_{13}H_9NOClBr$: N, 4·5; Cl and Br, 37·15 %.)

3:5-*Dibromosalicylideneaniline.* 3:5-Dibromosalicylaldehyde (m.p. 84° C.) was prepared by the method of Lindemann & Forth (1923).

The anil crystallized from 95 % ethanol in orange-red needles, m.p. 90·0–90·5° C. (Found: Br, 45·1. Calc. for $C_{13}H_9NOBr_2$: Br, 45·0 %.) Brewster (1924) gives m.p. 91° C. and Lindemann & Forth give m.p. 105° C.

3-*Bromo-5-methylsalicylideneaniline.* 3-Bromo-5-methylsalicylaldehyde was prepared from 3-bromo-*p*-cresol by the Reimer–Tiemann reaction, and crystallized from 90 % methanol as cream-coloured tabular needles, m.p. 65·0–65·5° C. (Found: Br, 36·6. Calc. for $C_8H_7O_2Br$: Br, 37·1 %.)

3-Bromo-5-methylsalicylideneaniline crystallized from 95 % ethanol as minute orange needles, m.p. 62° C. (softens at 60° C.). (Found: N, 4·9; C, 57·4; H, 4·15. Calc. for $C_{14}H_{12}NOBr$: N, 4·85; C, 57·95; H, 4·15 %.)

Copper 3-*chlorosalicylideneaniline.* Obtained by the reaction between cupric acetate and 3-chlorosalicylideneaniline in aqueous ethanol, m.p. 198–200° C. (Found: C, 59·2; H, 3·55. Calc. for $C_{26}H_{18}N_2O_2Cl_2Cu$: C, 59·4; H, 3·45 %.)

3-*Methoxysalicylideneaniline.* A commercial sample of *o*-vanillin was redistilled and condensed with aniline in hot ethanol. The product, recrystallized from 75 % ethanol, gave deep orange-red needles or prisms, m.p. 84·0–84·5° C. (Found: C, 73·4; H, 6·0. Calc. for $C_{14}H_{13}NO_2$: C, 74·0; H, 5·8 %.)

2-*Hydroxynaphthylideneaniline.* A recrystallized commercial sample of 2-hydroxy-1-naphthaldehyde was condensed with aniline in warm ethanol. The product crystallized from 70 % ethanol in deep golden-yellow needles, m.p. 93·0–93·5° C. (Found: N, 5·75. Calc. for $C_{17}H_{13}NO$: N, 5·65 %.)

3-*Chloro-4-hydroxybenzylideneaniline.* 3-Chloro-4-hydroxybenzaldehyde (m.p. 139° C.) was condensed with aniline in warm ethanol. The product crystallized from 50 % ethanol in glistening yellow-ochre needles, m.p. 167–168° C. (Found: C, 67·1; H, 4·5. Calc. for $C_{13}H_{10}NOCl$: C, 67·35; H, 4·35 %.)

Benzylidene-4-chloro-2-hydroxyaniline. 4-Chloro-2-hydroxyaniline (Hodgson & Kershaw, 1928) was condensed with benzaldehyde in warm ethanol. The product crystallized from 50% ethanol in silver-coloured plates, m.p. 108° C. (Found: Cl, 15·7. Calc. for $C_{13}H_{10}NOCl$: Cl, 15·3%.)

Formulation of experimental chemicals. Since 'ball-mill' grinding of these soft materials proved unsatisfactory, the required suspensions of all experimental compounds used in small-scale and large-scale trials were prepared by the repeated grinding of the moist sample in a mortar, with intermittent additions of aqueous methyl ethyl cellulose solution and decantation of this liquid, which contained the more finely divided material in suspension.

Activity against cucumber powdery mildew

Tests on pot plants

The procedure outlined in the first paper of this series (Smith & Read, 1961) was adopted. Estimations were made of eradicative activity against well-established, visible mildew, and also against incipient infection on the same plant assumed, by reference to control plants, to have been developing on certain younger leaves which showed no visible infection at the time of spray treatment. These visual estimations of the percentage leaf infection were made 1 week after spraying. Test plants had their lower leaves heavily infected with mildew, and were selected for uniformity of infection and growth; precise pretreatment estimate of infection on individual plants was therefore considered unnecessary. All compounds were tested on at least two occasions. Disease-control ratings were made as outlined in Paper I.

Trials in 1958 *on cucumber plants grown commercially*

In both trials spraying at high volume commenced when mildew began to appear on a number of plants, and was repeated at approximately 14-day intervals. All plants received normal commercial cultural treatment. The results of visual estimations of the percentage infection on the foliage of each plant are recorded in Tables 2 and 4.

Trial I. Cucumber plants were grown in the normal commercial manner in prepared beds in a standard 100 ft. cucumber house, and were given the four spray treatments listed below. Apart from treatment 2, which was applied to two plots only, each treatment was replicated four times on plots laid out at random in the 100 ft. glasshouse. All plots were separated from adjacent plots by 'guard' plants. It proved necessary to spray the control plots, previously unsprayed, three times, on 15 and 24 April and 6 May, to avoid the total loss of the plants. The restriction of treatment 2 was necessary owing to the cost of the laboratory synthesis of 3-bromosalicylideneaniline.

Treatments (applied in aqueous sprays).

(1) 3-*Chlorosalicylideneaniline* (0·10%), with methyl ethyl cellulose (0·04%) as dispersing and wetting agent.

(2) 3-*Bromosalicylideneaniline* (0·08%), with methyl ethyl cellulose (0·04%) as dispersing and wetting agent.

(3) 'Karathane WD', stated to contain 23% of 2:4-*dinitro*-6-(1-*methylheptyl*)*phenyl*

crotonate (see, however, the work of Kirby & Frick, 1958), at 8 oz./100 gal. (0·013% active material), with Manoxol OT/A (8 oz./100 gal.) as wetter.

(4) A *copper oxychloride/petroleum oil emulsion* mixture, giving 0·05% copper and 0·3% oil in the spray.

The effects of the treatments on mildew infection are given in Table 2, and the weight of the crop obtained under each treatment is given in Table 3.

Trial II. A formulation of 3-chlorosalicylideneaniline (0·10%), calcium carbonate (0·10%) and methyl ethyl cellulose (0·04%) was compared with 3-bromosalicylideneaniline and 'Karathane', both as used in trial I, and with a control (unsprayed).

The effects of the treatments on mildew infection are shown in Table 4. Owing to the necessarily shorter duration of this trial the crop records are of very limited value and are not presented.

Table 1. *Eradicative activity against cucumber mildew in small-scale tests on pot plants*

Compound	Fungicide conc. (%)	Disease-control index I = > 95% control II = 50–95% control
Salicylideneaniline	0·11*	I
	0·05	II
Salicylidene-4-chloroaniline	0·13	II
3-Chlorosalicylideneaniline	0·13	I
	0·06	I
3-Chlorosalicylidene-3-chloroaniline	0·14	II
3-Chlorosalicylidene-4-chloroaniline	0·14	II
4-Chlorosalicylideneaniline	0·13*	I
	0·03	II
5-Chlorosalicylideneaniline	0·13	II
3:5-Dichlorosalicylideneaniline	0·07*	I
	0·03	II
3-Chloro-5-methylsalicylideneaniline	0·14	I
	0·07	II
3-Bromosalicylideneaniline	0·14	I
	0·07	I
3-Bromo-5-chlorosalicylideneaniline	0·16	II
3:5-Dibromosalicylideneaniline	0·18	I
	0·09	II
3-Bromo-5-methylsalicylideneaniline	0·14*	I
	0·05	II

The following compounds were ineffective at 0·12–0·14% (or lower concentrations when phytotoxic at this strength): 3-chlorosalicylidene-2-chloroaniline, 5-chlorosalicylidene-4-chloroaniline, 3:5-dichlorosalicylidene-4-chloroaniline, 3-methoxysalicylideneaniline, copper 3-chlorosalicylideneaniline, 2-hydroxynaphthylideneaniline, 3-chloro-4-hydroxybenzylideneaniline and benzylidene-4-chloro-2-hydroxyaniline, and 3-chlorosalicylaldehyde (0·04%), 5-chlorosalicylaldehyde (0·04%) and 3:5-dichlorosalicylaldehyde (0·015%).

* = caused 'hardening' of foliage and reduction in growth rate.

Table 2. *Trial I. Control of cucumber mildew under commercial growing conditions (plants sprayed on 14 and 27 March, 15 and 24 April, 6 and 21 May, 4 June)*

Fungicide	Mean percentage area of foliage infected on					
	24 March	14 April	30 April	20 May	2 June	23 June
3-Chlorosalicylidene-aniline (0·10 %)	2·5	12·8	1·8	2·8	2·8	3·5
2:4-Dinitro-6-(1-methyl-heptyl)phenyl crotonate (0·013 %) ('Karathane')	6·0	39·5	14·3	6·0	4·0	3·8
Copper oxychloride/ petroleum emulsion (0·05 % Cu; 0·3 % oil)	3·0	28·0	3·5	2·8	1·5	2·3
Control (untreated)	52·0	61·0	*	*	*	*
Significant difference between means ($P = 0·05$)	11·2	6·4	9·9	4·2	4·7	10·7
3-Bromosalicylideneaniline (0·08 %)	0·5	4·5	1·5	1·5	1·0	1·3

* = Plants eliminated from experiment by severity of infection.

Table 3. *Effect of fungicidal treatments on yield of cucumber fruit in trial I*

Fungicide	Relative weights of cucumbers ('Karathane' = 100)
3-Chlorosalicylideneaniline (0·10 %)	84·0
'Karathane WD'	100·0
Copper oxychloride/petroleum emulsion	92·9
Significant difference between means ($P = 0·05$)	16·3
3-Bromosalicylideneaniline (0·08 %) (not replicated)	95·4

Table 4. *Trial II. Control of cucumber mildew under commercial growing conditions (plants sprayed on 6 and 19 August, 3 September)*

Fungicide	Mean percentage area of foliage infected on		
	18 Aug.	1 Sept.	15 Sept.
3-Chlorosalicylideneaniline (0·10 %) plus calcium carbonate (0·10 %)	1·5	11·3	12·8
3-Bromosalicylideneaniline (0·08 %)	0·5	2·8	4·3
'Karathane WD'	3·5	17·0	19·8
Control (untreated)	32·0	41·3	45·0
Significant difference between means ($P = 0·05$)	4·7	4·6	10·7

DISCUSSION

Small-scale tests

Salicylideneaniline showed appreciable eradicative activity against cucumber powdery mildew but was phytotoxic at a fully effective concentration. 3-Chloro- and 4-chloro-salicylideneaniline were effective at 0·13 %, at which concentration the

latter compound caused some hardening of the foliage, whereas 5-chlorosalicylideneaniline was inactive as an eradicant. However, the substitution of chlorine into the 5-position of 3-chlorosalicylideneaniline did not reduce anti-mildew activity, but caused a considerable increase in phytotoxicity, a factor *per se* capable of affecting the mildew.

The substitution of chlorine into the 4-positions of the anilo nuclei of salicylideneaniline and two monochlorinated derivatives resulted in a virtually complete loss of eradicative activity, and a tendency to reduce phytotoxicity. Similarly, chlorine in the 2- and 3-positions of the anilo nucleus of 3-chlorosalicylideneaniline eliminated anti-mildew activity.

Whereas the replacement of chlorine in 3-chlorosalicylideneaniline by bromine gave a small increase in anti-mildew activity, the 3- or 3:5-replacement of chlorine in 3:5-dichlorosalicylideneaniline by bromine gave no increase in anti-mildew activity. Such replacements did, however, decrease phytotoxicity. The substitution of methyl groups into the 5-positions of the two most promising compounds, 3-chloro- and 3-bromo-salicylideneaniline, did not enhance their eradicative action.

The methoxy group, reputedly isosteric with chlorine, cannot successfully replace the chlorine atom in 3-chlorosalicylideneaniline, for 3-methoxysalicylideneaniline proved to be virtually inactive.

The replacement of the salicylidene group of salicylideneaniline by 2-hydroxynaphthylidene caused a pronounced fall in anti-mildew and phytotoxic activities.

None of the less closely related compounds, or the parent chlorinated salicylaldehydes, showed any appreciable fungicidal activity at non-phytotoxic concentrations. For example, 3-chloro-4-hydroxybenzylideneaniline was quite inactive, and the reversal of the bridging azomethine group of the active 4-chlorosalicylideneaniline, giving benzylidene-4-chloro-2-hydroxyaniline, caused a virtual loss of toxicity towards well-established mildew.

Commercial-scale trials

During trial I it became apparent that although 3-chlorosalicylideneaniline gave a control of mildew equal to, or possibly better than that obtained in plots treated with standard 'Karathane' or standard copper oxychloride/petroleum emulsion sprays it had a delayed deleterious effect on the vigour of the plants. This phytotoxic effect, noticeable after four successive spray treatments over a period of 6 weeks, reduced growth rate and total leaf area, and gave a 16% reduction in crop yield, compared with those from 'Karathane'-sprayed plots, which was just significant at 5% probability. Furthermore, applications of 3-chloro- and 3-bromo-salicylideneaniline on 6 May both caused considerable leaf scorch. No apparent injury was caused by the other six sprayings with the 3-bromo analogue, and the reason for this isolated occurrence of leaf scorch remains obscure. However, apart from this single occurrence of phytotoxicity, which would appear to preclude its use on cucumber crops, at least in the formulation used in trial I, the 3-bromo compound gave a very good control of a severe infection of powdery mildew with no apparent reduction in yield in comparison with yields from plots treated with the two established fungicides.

In trial II, 3-bromosalicylideneaniline, at 0·08%, again gave a control of severe

powdery mildew significantly superior to that given by the standard 'Karathane' treatment, without causing any apparent phytotoxicity or crop reduction (relative to the 'Karathane'-treated plots) during the shorter period of this trial. Calcium carbonate (0·1%) was incorporated in the 3-chlorosalicylideneaniline spray as preliminary tests on pot plants had shown that calcium carbonate, one of a number of additives tested, reduced the hardening effect of this compound at 0·25%. However, 3-chlorosalicylideneaniline, at 0·10%, although formulated with calcium carbonate (0·10%), again caused a cumulative phytotoxicity to plants in the border and was significantly inferior to 3-bromosalicylideneaniline in controlling mildew.

No abnormal flavour was detected when cucumbers sprayed with these experimental compounds were sampled by a small group of tasters.

APPENDIX

Toxicity to other pathogenic fungi

The *in vitro* toxicities of 3-chloro- and 3-bromo-salicylideneaniline towards spores (*c.* 100,000/1 ml.) of tomato leaf-mould (*Cladosporium fulvum* Cooke), when plotted on logarithmic-probability paper, show (Table 5) that their fungitoxic activities can equal, or surpass that of salicylanilide.

Table 5. In vitro *fungitoxicity to spores of* Cladosporium fulvum *Cooke*

Compound	ED 50 (in parts per million)	ED 95 (in parts per million)
Salicylanilide	4·2	41·0
3-Bromosalicylideneaniline	4·7	12·5
3-Chlorosalicylideneaniline	3·9	35·0

In preliminary tests these two experimental compounds also showed marked eradicative activity against gooseberry powdery mildew (*Sphaerotheca mors-uvae* (Schw.) Berk.), chrysanthemum mildew (*Oidium chrysanthemi* Rabenh.) and rose mildew (*Sphaerotheca pannosa* (Wallr.) Lev.), without apparent phytotoxicity.

Both compounds were, however, phytotoxic to strawberry plants (var. 'Royal Sovereign'), which showed leaf-scorch after a single spray treatment, and caused a delayed stunting of tomato plants (var. 'Ailsa Craig'). Also, while apparently non-phytotoxic *per se* to gooseberry plants, the 3-chloro analogue was incompatible with DDT on the varieties 'Whinham's Industry' and 'Leveller'. Although 3-bromosalicylideneaniline, the most promising compound, possesses a high order of eradicative activity against certain powdery mildews, it is less safe for use on several host plants than two of the fungicides now in use against these fungi and, since it caused damage to cucumber plants on one occasion (6 May), its safety on any other crop could not be established without extensive trials.

Tests at East Malling showed that several compounds of this series were not as effective as 'Karathane' against apple mildew (*Podosphaera leucotricha*) at low concentrations; the effects of higher concentrations (0·07–0·10%) are not known.

No appreciable acaricidal activity against the red spider mite (*Tetranychus urticae*)

on cucumber and tomato plants was shown by compounds of this series, although 3:5-dichlorosalicylideneaniline appeared to irritate and repel the mite.

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Investigations on fungitoxic derivatives of salicylaldehyde

III. Some N-substituted derivatives of halogenated salicylideneimines as eradicants of cucumber powdery mildew

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SUMMARY

Certain N-alkyl, N-aryl and N-heterocyclic derivatives of three halogenated salicylideneimines have been synthesized and tested for eradicative activity against cucumber powdery mildew. Although the activity of several of these compounds closely approached that of 3-chlorosalicylideneaniline, which showed pronounced activity in an earlier investigation, no compound of practical value was discovered.

INTRODUCTION

Several halogenated derivatives of salicylideneaniline, and especially 3-bromosalicylideneaniline and 3-chlorosalicylideneaniline, have shown considerable eradicative activity against cucumber powdery mildew (*Erysiphe cichoracearum* DC.) but appear to be too phytotoxic for commercial use (Smith & Read, 1961*b*).

A series of closely related compounds having N-substituents other than phenyl was therefore investigated, in the attempt to discover one that would be as effective, or more effective, against powdery mildews, and non-phytotoxic. Model & Bindler (1958) reported that the N-amyl derivatives of 5-chloro- and 3:5-dichlorosalicylamide were more fungitoxic than the N-phenyl derivatives of these two compounds.

EXPERIMENTAL

Chemicals

The experimental compounds were prepared by the condensation of the appropriate halogenated derivative of salicylaldehyde with the appropriate amino compound, in warm ethanol. The product was precipitated by cooling, usually after the addition of water, and was recrystallized to constant melting point, or, if liquid at room temperature, was purified by distillation under reduced pressure. Microanalyses were carried out by Drs Weiler and Strauss, of Oxford.

3-*Chlorosalicylideneethylamine*. Crystallized from light petrol in yellow microcrystals, m.p. 27·5–28·0° C. (Found: C, 58·8; H, 5·3. Calc. for $C_9H_{10}NOCl$: C, 58·8; H, 5·5 %.)

3-*Chlorosalicylidene-2-bromoethylamine*. Crystallized from ethanol in long, flat aggregations of lemon-yellow crystals, m.p. 63·5° C. (Found: Cl and Br, 43·5; C, 41·4; H, 3·4. Calc. for $C_9H_9NOClBr$: Cl and Br, 43·9; C, 41·2; H, 3·4 %.)

3-*Chlorosalicylideneallylamine.* The solvent was removed, under reduced pressure, and the liquid residue was dissolved in ether. The ethereal solution was extracted several times with water, dried over anhydrous magnesium sulphate and filtered. Removal of the ether gave an orange-coloured liquid (Found: C, 61·2; H, 4·9. Calc. for $C_{10}H_{10}NOCl$: C, 61·35; H, 5·15 %) which was unstable when distilled *in vacuo*.

3-*Chlorosalicylaldehyde semicarbazone.* Prepared by the standard method, then finely powdered and purified by the extraction of impurities with boiling ethanol. White microcrystals, m.p. 252° C., were obtained. (Found: N, 19·5. Calc. for $C_8H_8N_3O_2Cl$: N, 19·7 %.)

3-*Chlorosalicylidenecyclohexylamine.* The alcoholic reaction mixture was dried over anhydrous magnesium sulphate and filtered. The filtrate was distilled under reduced pressure to remove the solvent, and the required product was obtained as a golden-yellow liquid, b.p. 152° C./0·5 mm. (Found: C, 66·0; H, 6·7; Cl, 15·3. Calc. for $C_{13}H_{15}NOCl$: C, 66·0; H, 6·4; Cl, 15·0 %.)

3-*Chlorosalicylidenefurfurylamine.* Crystallized from 90 % ethanol in flat lemon-yellow needles, m.p. 58° C. (Found: C, 60·6; H, 4·3; Cl, 15·3. Calc. for $C_{12}H_{10}NO_2Cl$: C, 61·1; H, 4·3; Cl, 15·05 %.)

3-*Chlorosalicylidene-2-aminothiazole.* Crystallized from ethanol in pale orange-yellow needles, m.p. 116·0–116·5° C. (Found: C, 49·7; H, 3·05; N, 11·7. Calc. for $C_{10}H_7N_2SOCl$: C, 50·3; H, 2·95; N, 11·75 %.)

3-*Chlorosalicylidene-2-aminopyridine.* Crystallized from 65 % ethanol in orange microprisms, m.p. 99·0–99·5° C. (Found: C, 61·6; H, 4·3. Calc. for $C_{12}H_9N_2OCl$: C, 61·9; H, 3·9 %.)

3-*Chlorosalicylidene-3-amino-1:2:4-triazole.* Crystallized from 90 % ethanol in yellow needles, m.p. 224° C. (Found: N, 24·8. Calc. for $C_9H_7N_4OCl$: N, 25·2 %.)

3-*Chlorosalicylidenebenzylamine.* Crystallized from 80 % ethanol in lemon-yellow needles, m.p. 62·5–63·5° C. (Found: C, 68·2; H, 5·0. Calc. for $C_{14}H_{12}NOCl$: C, 68·4; H, 4·9 %.)

3-*Chlorosalicylaldehydephenylhydrazone.* Crystallized from 70 % ethanol in pale yellow-brown platelets, m.p. 119–120° C. (Found: N, 11·0. Calc. for $C_{13}H_{11}N_2OCl$: N, 11·35 %.)

3-*Chlorosalicylideneaniline.* Crystallized from ethanol in short, pale-orange needles, m.p. 59·5–60·0° C. (Found: C, 68·0; H, 4·25; N, 6·4; Cl, 15·1. Calc. for $C_{13}H_{10}NOCl$: C, 67·35; H, 4·35; N, 6·05; Cl, 15·3 %.)

3-*Chlorosalicylidene-p-toluidine.* Crystallized from 80 % ethanol in small, orange-yellow platelets, m.p. 99·5° C. (Found: C, 69·1; H, 5·0. Calc. for $C_{14}H_{12}NOCl$: C, 68·4; H, 4·9 %.)

3-*Chlorosalicylidene-4-nitroaniline.* Crystallized from benzene in minute orange-pink platelets, m.p. 177° C. (Found: C, 56·9; H, 2·9. Calc. for $C_{13}H_9N_2O_3Cl$: C, 56·4; H, 3·25 %.)

3-*Chlorosalicylidene-2-carboxyaniline.* Crystallized from aqueous acetic acid in orange-scarlet microcrystals, m.p. 204–205° C. (Found: Cl, 13·4. Calc. for $C_{14}H_9NO_3Cl$: Cl, 12·9 %.)

3-*Chlorosalicylidene-1-naphthylamine.* Crystallized from ethanol in very fine lemon-yellow needles, m.p. 101° C. (Found: C, 72·6; H, 4·3. Calc. for $C_{17}H_{12}NOCl$: C, 72·45; H, 4·3 %.)

3-*Chlorosalicylidene azine.* Prepared by the standard method. The product crystallized from 95 % acetic acid in small, dull-yellow needles, m.p. 250° C. (Found: N, 9·45. Calc. for $C_{14}H_{10}N_2O_2Cl_2$: N, 9·05 %.)

3:5-*Dichlorosalicylideneethylamine.* Crystallized from ethanol in deep lemon-yellow plates, m.p. 70·5–71·0° C. (Found: Cl, 32·5; N, 6·7. Calc. for $C_9H_9NOCl_2$: Cl, 32·55; N, 6·4 %.)

3:5-*Dichlorosalicylidene-2-bromoethylamine.* Crystallized from 75 % ethanol in deep-yellow microcrystals, m.p. 75·5° C. (Found: N, 4·8. Calc. for $C_9H_8NOBrCl_2$: N, 4·75 %.)

3:5-*Dichlorosalicylideneallylamine.* Crystallized from light petrol in golden-yellow needles or prisms, m.p. 56° C. (Found: C, 52·4; H, 4·1. Calc. for $C_{10}H_9NOCl_2$: C, 52·15; H, 3·95 %.)

3:5-*Dichlorosalicylidene-n-heptylamine.* Alcohol was removed from the reaction mixture by distillation under reduced pressure. The liquid residue was dissolved in ether, dried over anhydrous magnesium sulphate and filtered. After removal of the ether, the required product was obtained as an orange-yellow liquid by distillation, b.p. 228° C./13 mm. (Found: C, 58·45; H, 6·8. Calc. for $C_{14}H_{19}NOCl_2$: C, 58·3; H, 6·65 %.)

3:5-*Dichlorosalicylidenebenzylamine.* The product crystallized from 75 % ethanol in golden-yellow needles, m.p. 100·5° C. (Found: C, 59·9; H, 3·8. Calc. for $C_{14}H_{11}NOCl_2$: C, 60·0; H, 3·95 %.)

3-*Bromosalicylidenebenzylamine*. Crystallized from 90% ethanol in lemon-yellow plates, m.p. 72·5–73·0° C. (Found: C, 57·7; H, 4·3. Calc. for $C_{14}H_{12}NOBr$: C, 57·9; H, 4·15%.)

3-*Bromosalicylidene-2-aminothiazole*. Crystallized from 70% ethanol in pale yellow-ochre plates, m.p. 126° C. (Found: Br, 28·4. Calc. for $C_{10}H_7NSOBr$: Br, 28·25%.)

Assessment of anti-mildew activity

The procedure adopted in testing these compounds against cucumber powdery mildew was outlined in earlier papers (Smith & Read, 1961*a*, *b*).

Table 1. *Eradicative activity against cucumber mildew*

Compound	Fungicide conc. (%)	Disease-control index I = > 95% control II = 50–95% control
3-Chlorosalicylideneethylamine	0·06*	II
	0·03	II
3-Chlorosalicylidene-2-bromoethylamine	0·14	I
	0·07	II
3-Chlorosalicylideneallylamine	0·10*	I
	0·05	II
3-Chlorosalicylidene*cyclo*hexylamine	0·04*	II
3-Chlorosalicylidenefurfurylamine	0·12	I
	0·06	II
3-Chlorosalicylidene-2-aminothiazole	0·12	I
	0·06	II
3-Chlorosalicylidene-2-aminopyridine	0·12*	II
3-Chlorosalicylidenebenzylamine	0·14	I
	0·07	II
3-Chlorosalicylidene-*p*-toluidine	0·14	II
3-Bromosalicylidenebenzylamine	0·14	I
	0·07	II
3-Bromosalicylidene-2-aminothiazole	0·14*	I
	0·07	II
3:5-Dichlorosalicylideneethylamine	0·05*	I
	0·015	II
3:5-Dichlorosalicylidene-2-bromoethylamine	0·10	I
	0·05	II
3:5-Dichlorosalicylideneallylamine	0·03*	II
3:5-Dichlorosalicylidene-*n*-heptylamine	0·03*	II
3:5-Dichlorosalicylidenebenzylamine	0·14	II

The following compounds (at 0·12–0·16%) were ineffective: 3-chlorosalicylidene-4-nitroaniline, 3-chlorosalicylidene-2-carboxyaniline, 3-chlorosalicylidene-1-naphthylamine, 3-chlorosalicylidene-3-amino-1:2:4-triazole, 3-chlorosalicylaldehyde phenylhydrazone, 3-chlorosalicylaldehyde semicarbazone and 3-chlorosalicylidene azine.

Mean control infection ranged between 50–70%.

* = phytotoxic.

DISCUSSION

The N-ethyl, N-2-bromoethyl and N-allyl derivatives of 3-chlorosalicylideneimine and 3:5-dichlorosalicylideneimine, and the N-heptyl derivative of the latter compound, showed slight to moderate activities against well-established cucumber mildew at

non-phytotoxic concentrations. These derivatives did, however, with one exception, appear to prevent the development of the 10–20% incipient fungal infection which, on the basis of evidence provided by the control plants, was almost certainly present at the time of spraying. Whereas the above N-ethyl derivatives were somewhat phytotoxic, the substitution of bromine into the 2-positions of the N-ethyl groups caused definite reductions in phytotoxicity. Another unicyclic compound, 3-chlorosalicylaldehyde semicarbazone, was quite inactive.

A number of the derivatives containing two bridged-ring structures were more fungicidal, and less phytotoxic, than the unicyclic derivatives examined, with the notable exception of 3-chlorosalicylidene*cyclo*hexylamine, which caused considerable leaf-scorch. Of the bicyclic derivatives of 3-chlorosalicylideneimine only the N-benzyl, N-furfuryl and N-thiazolyl (thiazyl) derivatives showed pronounced activity at 0·12%–0·14%, but these were not as active as the N-phenyl derivative, 3-chlorosalicylideneaniline. 3-Bromosalicylidenebenzylamine also showed moderate activity, whereas 3:5-dichlorosalicylidenebenzylamine, unlike 3:5-dichlorosalicylideneaniline which was found to be both fungicidal and phytotoxic (Smith & Read, 1961*b*), was virtually inactive against well-established mildew and was non-phytotoxic at 0·14%. 3-Bromosalicylidene-2-aminothiazole was moderately active at 0·14% but was phytotoxic, unlike the 3-chloro analogue.

The substitution of a methyl or a nitro group into the 4-position, or a carboxy group into the 2-position of the phenylimino nucleus of 3-chlorosalicylideneaniline reduced activity very considerably (certain N-substituted derivatives of *ortho*-aminobenzoic acid have shown some activity against this disease (Rich & Horsfall, 1957)), as did the replacement of phenylimino by the larger naphthylimino nucleus.

A molecule of somewhat different type, 3-chlorosalicylidene azine, formed by the N:N union of two 3-chlorosalicylideneimine molecules, showed no activity.

Taking into account results in this, and earlier (Smith & Read, 1961*b*) work, it is clear that the 3-chloro-, 3-bromo- and 3:5-dichloro-salicylideneimino groups confer activity towards cucumber powdery mildew only when combined with a suitable radical, such as phenyl.

We wish to thank Miss B. M. Finch for assistance during this work.

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The fungitoxicity of metal ions

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SUMMARY

The *in vitro* fungistatic activity of some twenty-four metal cations has been determined against *Alternaria tenuis* and *Botrytis fabae*. The metal salts, mainly nitrates, were tested in aqueous solution without added spore germination stimulant. The logarithm of the metal ion concentration at the ED 50 value has been found to conform to the exponential relationship with electronegativity proposed by Danielli & Davies (1951). These results are discussed in relation to the site of action of metal cations on the fungal cell.

INTRODUCTION

The concept of a general relation between metal ion toxicity and some physical or chemical property of the metal has held interest since the beginning of this century. Mathews (1904) considered the toxicity of metal ions to eggs of the fish *Fundulus heteroclitus* to be related to the ease with which the ions gained or lost electric charges and hence to the electrode potential of the metal. Later work by Jones (1939, 1940) has shown that for a given degree of toxic action on the stickleback, *Gasterosteus aculeatus*, and the planarian, *Polycelis nigra*, the logarithm of the toxic concentration of some eighteen metal ions is a linear function of the standard electrode potential. This hypothesis has been criticized by Danielli & Davies (1951) who point out that the slopes of Jones's plots are in poor agreement with the predictions of the Nernst formulae in which observed electrode potential should increase by 0·06 V. for a tenfold rise in concentration of a univalent ion, whereas the observed increase is 1·0 V. Danielli & Davies consider that the tightness of covalent binding of the ion to ionogenic groups at the cell surface is primarily responsible for the toxicity of metal ions, rather than oxidation by the ions, and they have proposed the electronegativity value of the metal as a measure of this chemical reaction. From a consideration of the energy of covalent bond formation between a metal ion and an oxygen-containing group on the cell surface these authors have derived an exponential relationship between the logarithm of the toxic concentration of the metal ion and its electronegativity value. Danielli & Davies have shown that this relationship gives fair agreement with the toxicity data of Jones (1940).

Another quantitative approach, although only applicable to the heavy metals, is that of Shaw (1954) who suggested that metal ion toxicity was caused by reaction of the cations with sulphydryl groups on the catalytically active site of the key enzyme, the driving force of this reaction being proportional to the insolubility of the metal sulphides. Shaw applied this principle to data in the literature and gave a correlation

of toxicity with sulphide insolubility for the heavy metals. However, Lowry, Sussman & von Böventer (1957) have found that although the toxicity of some seven metal cations to *Neurospora* ascospores varied directly as a function of the insolubility of their mercaptides the slope of the curve did not agree with that given by Shaw for other organisms.

As some of the most important fungicides are metal salts there is especial interest in a general relationship governing the fungitoxicity of metal ions. However, except for the studies of Lowry *et al.* (1957), previous work in this field has been only of a qualitative nature. Seifriz (1949) has considered the toxicity of metal ions to the slime mould *Myxomycetes* in terms of hydration and mobility for the lighter metal ions and electronegativity for the noble metals. From a review of the literature, Horsfall (1956) has observed that there is a good correlation between the fungitoxicity of metals and their ability to form stable chelate compounds. The most comprehensive study of metal ion fungitoxicity is that by McCallan & Wilcoxon (1934) who tested the majority of the elements in the Periodic Table against four species of fungi. However, their data on two of the fungi are vitiated by the use of orange juice as a spore germination stimulant, a material known (Horsfall, 1956) to complex with metal ions and reduce their toxicity.

To determine whether the general relationship of Danielli & Davies (1951) can be applied to the fungitoxicity of metal ions, solutions of a wide range of metal ions were tested against the conidia of two species of fungi. The species chosen gave high percentage germination in distilled water so that spore stimulants were not required. As far as possible all the salts possessed the common nitrate anion, and the toxicities of the less toxic metals, which were not given by McCallan & Wilcoxon (1934), were measured.

Preliminary results of this investigation have already been reported (Somers, 1959, 1960).

MATERIALS AND METHODS

Fungi. Conidia were washed from 7-day-old cultures of *Alternaria tenuis* Nees and *Botrytis fabae* Sardiña, grown on malt agar and a liquid dextrose medium, respectively. The spores were filtered through cheesecloth, washed three times by centrifugation, then suspended in distilled water.

Fungicides. The compounds used were of laboratory reagent grade; all were made up in freshly distilled water.

Measurement of fungitoxicity. A test-tube dilution spore germination technique, as recommended by the American Phytopathological Society Committee on Standardization of Fungicidal Tests (1943, 1947), was used for the evaluation of *in vitro* fungistatic activity. Aqueous solutions of the fungicides containing spores at 50,000/ml. were pipetted on to glass slides (covered with a baked film of cellulose acetate), incubated in a moist chamber at 25° C., and the spore germination recorded after 18 hours. In control experiments *A. tenuis* and *B. fabae* invariably gave 98–100% germination in distilled water.

RESULTS

Fungitoxicity of the metal salts

The median effective doses, ED 50, of the metal salts given in Table 1 were determined by plotting the probit of percentage inhibition of germination against the logarithm of dose and visually fitting the best line. As the aim of these experiments was to compare toxicity values that range from 10^{0} to 10^{-7} a precise determination of the ED 50 was considered unnecessary. Each series of experiments measured the germination response of some five or six compounds and included a bioassay of copper sulphate as a standard. The extent of day-to-day variation of response of the fungi to the copper sulphate was slight in comparison to the range of toxicities investigated; thus against *A. tenuis* the mean ED 50 was $5{\cdot}74 \times 10^{-6}$ moles/l. with S.E. $= 0{\cdot}65$ (16 determinations), and for *B. fabae* the ED 50 was $8{\cdot}52 \times 10^{-6}$ with S.E. $= 0{\cdot}44$ (15 determinations). All the ED 50 values given in Table 1 are the means from at least three separate experiments; some of these values differ slightly from the provisional ones given earlier (Somers, 1959, 1960). It should be noted that the logarithmic scale previously used for *A. tenuis* (Somers, 1959) was an arbitrary one and that the toxicity of copper was erroneously plotted too low.

Table 1. *Fungitoxicity of metal salts to* Alternaria tenuis *and* Botrytis fabae

Salt	Metal ion	ED 50 in moles/l. of metal ion		pH at ED 50	
		A. tenuis	*B. fabae*	*A. tenuis*	*B. fabae*
Aluminium sulphate	Al^{3+}	$1{\cdot}3 \times 10^{-5}$	$1{\cdot}4 \times 10^{-4}$	5·0	4·4
Barium nitrate	Ba^{2+}	$1{\cdot}6 \times 10^{-1}$	$(3{\cdot}8 \times 10^{-1})$*	5·6	5·4
Beryllium nitrate	Be^{2+}	$6{\cdot}0 \times 10^{-5}$	$4{\cdot}4 \times 10^{-4}$	5·4	5·1
Calcium nitrate	Ca^{2+}	$2{\cdot}0 \times 10^{-1}$	$2{\cdot}0 \times 10^{-1}$	5·9	5·9
Chromic nitrate	Cr^{3+}	$4{\cdot}5 \times 10^{-6}$	$1{\cdot}25 \times 10^{-5}$	5·8	5·8
Cobalt nitrate	Co^{2+}	$6{\cdot}5 \times 10^{-3}$	$2{\cdot}85 \times 10^{-4}$	6·2	6·9
Cupric nitrate	Cu^{2+}	$4{\cdot}6 \times 10^{-6}$	$1{\cdot}0 \times 10^{-5}$	6·9	6·1
Lead nitrate	Pb^{2+}	$1{\cdot}1 \times 10^{-5}$	$2{\cdot}2 \times 10^{-5}$	6·2	6·0
Lithium nitrate	Li^{+}	$2{\cdot}4 \times 10^{-1}$	$1{\cdot}9 \times 10^{-2}$	6·7	6·2
Magnesium nitrate	Mg^{2+}	$2{\cdot}7 \times 10^{-1}$	$2{\cdot}1 \times 10^{-1}$	3·5	3·9
Manganese nitrate	Mn^{2+}	$1{\cdot}9 \times 10^{-2}$	$2{\cdot}1 \times 10^{-2}$	2·7	2·2
Mercuric chloride	Hg^{2+}	$5{\cdot}5 \times 10^{-6}$	$5{\cdot}5 \times 10^{-6}$	8·0	7·3
Mercurous chloride	Hg^{+}	$3{\cdot}4 \times 10^{-6}$	$3{\cdot}5 \times 10^{-6}$	7·6	7·1
Nickel nitrate	Ni^{2+}	$1{\cdot}1 \times 10^{-3}$	$8{\cdot}6 \times 10^{-5}$	6·1	7·1
Osmium tetroxide	Os^{4+}	$1{\cdot}7 \times 10^{-7}$	$2{\cdot}4 \times 10^{-7}$	6·8	6·2
Palladium nitrate	Pd^{2+}	$1{\cdot}1 \times 10^{-4}$	$3{\cdot}5 \times 10^{-5}$	5·2	5·6
Potassium nitrate	K^{+}	$1{\cdot}3$	$3{\cdot}6 \times 10^{-1}$	6·6	6·2
Ruthenium trichloride	Ru^{3+}	$1{\cdot}6 \times 10^{-5}$	$5{\cdot}3 \times 10^{-5}$	6·3	5·8
Silver nitrate	Ag^{+}	$7{\cdot}6 \times 10^{-7}$	$1{\cdot}3 \times 10^{-6}$	7·2	7·1
Sodium nitrate	Na^{+}	$4{\cdot}6 \times 10^{-1}$	$1{\cdot}6 \times 10^{-1}$	6·5	6·5
Strontium nitrate	Sr^{2+}	$3{\cdot}4 \times 10^{-1}$	$1{\cdot}5 \times 10^{-1}$	6·3	5·6
Thallous nitrate	Tl^{+}	$2{\cdot}9 \times 10^{-4}$	$8{\cdot}6 \times 10^{-5}$	6·7	6·5
Yttrium nitrate	Y^{3+}	$6{\cdot}3 \times 10^{-6}$	$1{\cdot}0 \times 10^{-5}$	6·9	6·9
Zinc nitrate	Zn^{2+}	$5{\cdot}4 \times 10^{-2}$	$1{\cdot}2 \times 10^{-3}$	3·7	5·1

* Extrapolated from dilute concentration.

In order to simplify the comparisons of toxicity the metal salts were tested as nitrates when possible. For aluminium the sulphate was more convenient to handle

than the hygroscopic nitrate, whilst for mercury the chlorides were used instead of the nitrates, which hydrolyse to basic salts. Although both palladium nitrate and ruthenium trichloride decomposed slightly in aqueous solution there was no microscopic evidence of precipitation by these compounds at their ED 50 concentrations.

The pH of the metal salt/spore suspension (50,000/ml.) at the ED 50 concentration was measured (Table 1); addition of the spore suspension to the metal salt increased the pH by approximately 0·1 unit. As the pH of the spore suspensions alone in distilled water was 6·4, only the salts of aluminium, magnesium, manganese, and zinc were appreciably hydrolysed at the concentrations tested.

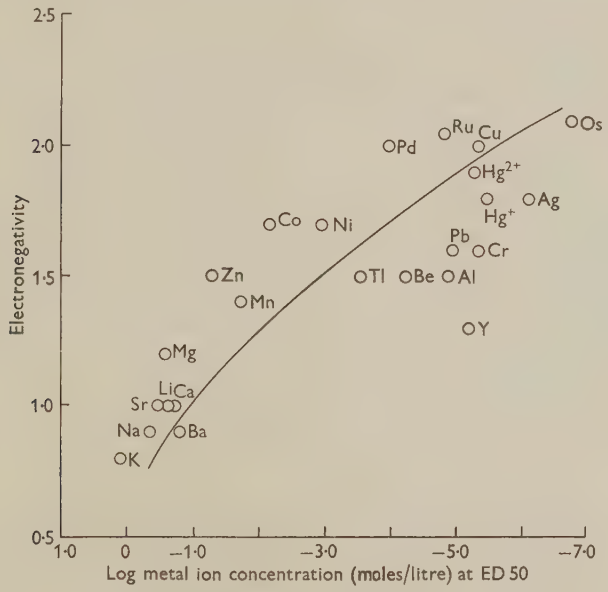

Fig. 1. Plot of the toxicity of metal cations to *Alternaria tenuis* against the electronegativity of the metal.

There is no evidence from Table 1 that either of the two fungi is consistently more susceptible than the other to the range of metals tested. Those metals which are more toxic to one species than the other tend to have similar chemical and physical properties. Thus aluminium, beryllium, and yttrium, and copper and silver, are more toxic to *A. tenuis* than to *B. fabae*, whilst the opposite behaviour is shown by the group 1 elements lithium, sodium, and potassium and the analogous thallous ion.

Adsorption of metal ions by glass

As low concentrations of metal ions are used in bioassay the nominal concentration of the ions in the bulk phase can be appreciably reduced by adsorption on the surface of the glass containing vessels. The effects will be most marked with the heavy metals especially in neutral solution. McIntosh (1959) found that a solution of mercuric

chloride at 60 p.p.m. Hg decreased in strength by 25% when diluted in three stages, in soda-glass tubes, to 1 p.p.m.

To determine the extent to which adsorption on glass affects the ED 50 values in Table 1, solutions of salts of the representative heavy metals, mercuric chloride and copper and silver nitrate, were diluted in borosilicate glass flasks and test-tubes to their ED 50 concentrations and then analysed chemically. The metal ions were determined colorimetrically, mercury and silver with dithizone (Sandell, 1944) and copper

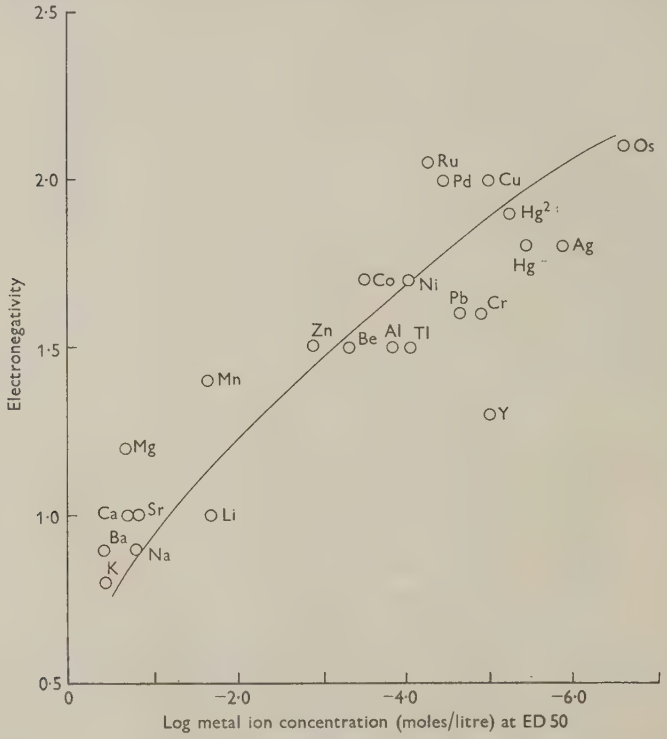

Fig. 2. Plot of the toxicity of metal cations to *Botrytis fabae* against the electronegativity of the metal.

with bis-*cyclo*-hexanone oxalyldihydrazone (Somers & Garraway, 1957). Using the same dilution technique as had been applied for the bioassays the loss in strength of the metal ions at the ED 50 concentrations was 18% for silver nitrate, 16% for mercuric chloride, and 6% for copper nitrate. The relative error in ED 50 values for these metals, which are those expected to show the greatest adsorption on glass, is negligible in terms of the wide range of fungitoxicities investigated. In fact, if the values of the log ED 50 for silver in Figs. 1 and 2 are corrected for loss in solution strength the revised values still lie within the perimeter of the circular symbols used in the figures.

Application of Danielli & Davies's hypothesis to the fungitoxicity data

Danielli & Davies (1951) have derived the following relationship between electronegativity and toxicity

$$\log [M^{q+}] = \alpha - \gamma e^{-0.25x^2}, \qquad (1)$$

where $[M^{q+}]$ is the concentration of the metal cation, of valency q, in the bulk phase which gives the standard toxicity; x is the difference between the electronegativity of the metal and that of oxygen; α and γ are constants. Danielli & Davies have taken x as a measure of the ionic character of the links between metal and cell surface and then have derived ΔG, the covalent free energy of formation of the bond between metal ion and oxygen. For the reaction: $AM^{q+-1} \rightleftharpoons M^{q+} + A^{-}$, where A is the oxygen-containing group of the surface, the equilibrium constant, K, is related to ΔG by $\log K = -\Delta G/2{\cdot}3RT$. Thus equation (1) can be evaluated, assuming that the fraction of the cell surface covered by insoluble complex is constant at equal toxicities.

From the results in Table 1, $\log [M^{q+}]$ for the two fungi was plotted against $e^{-0.25x^2}$ and the regression lines calculated, giving $\alpha = 1{\cdot}68$ (S.E. 0·77) and $\gamma = 12{\cdot}95$ (S.E. 1·92) for *A. tenuis*, and $\alpha = 1{\cdot}22$ (S.E. 0·59) and $\gamma = 12{\cdot}09$ (S.E. 1·46) for *B. fabae*. The original electronegativity scale given by Pauling (1948) includes values for only a few of the metals tested; the other electronegativities that have been used are those calculated by Haïssinsky (1946) by the same method as Pauling for the whole Periodic Table including values for some of the different valence states. Although many different methods for the determination of electronegativities have been proposed, recent work largely confirms Pauling's original calculations (Howlett, 1959). The electronegativities are plotted against the logarithms of the ED 50 values of the metal ions ($\log[M^{q+}]$) for *A. tenuis* in Fig. 1 and for *B. fabae* in Fig. 2; the calculated values of α and γ given above being used to plot the curves.

Examination of Figs. 1 and 2 and of the calculated values of α and γ shows that equation (1) gives a reasonably good fit to the experimental results and there is no significant difference in the equation for the two fungi. Yttrium occupies an anomalous position with both fungi and there is a wider scatter of results with *A. tenuis* than *B. fabae* but, nevertheless, the general relationship proposed by Danielli & Davies does appear to be valid for the wide range of fungitoxicity investigated.

DISCUSSION

One of the observations which has sustained the belief in a relationship between the toxicity of metal ions and their physico-chemical properties is that there is a general order of metal toxicity independent of the organism tested. From an extensive review of the literature Horsfall (1956) has found the following order of descending fungitoxicity to apply to a range of fungal species:

$$\text{Ag} > \text{Hg} > \text{Cu} > \text{Cr} > \text{Ni} > \text{Pb} > \text{Co} > \text{Zn} > \text{Ca},$$

with the proviso that the position of nickel is variable. In fact, with an interchange of positions of lead and nickel, this is the same order of toxicity to *A. tenuis* and *B. fabae* as shown in the present experiments.

Although the electronegativity hypothesis of Danielli & Davies (1951) gives reasonable agreement with the fungitoxicity data the variability in the known electronegativity values precludes more exact correlation. Howlett (1959) has pointed out that as electronegativity is better considered as an orbital property than an atomic one many elements should not be assigned a single electronegativity value but rather a range of effective values. When Danielli & Davies applied equation (1) to the toxicity data against *Polycelis nigra*, α and γ were found to equal 0·95 and 9·28, respectively; values that are of the same order as those given above for *A. tenuis* and *B. fabae*, although the different organisms may be expected to contain different reactive surfaces.

These results suggest that the fungistatic action of metal cations is related to their strength of covalent binding to surface ionogenic groups of the cell, i.e. to such groups as imidazole, carboxyl, phosphate, or sulphydryl. The site of these reactive groups is probably outside or on the cytoplasmic membrane, for much of the experimental work on micro-organisms has shown the initial uptake of cations to be a surface phenomenon (Cochrane, 1958). Extensive studies on yeast by Rothstein (1959) have identified the anionic receptor sites for heavy metals as being at or near the cell surface, external to the permeability barrier; phosphoryl and carboxyl groups are considered possible binding sites (Rothstein & Hayes, 1956). Similarly, Lowry *et al.* (1957) have suggested that metal cations are adsorbed on to the surface of *Neurospora* ascospores and do not gain access to sensitive receptor sites until germination begins. Further work (Sussman, von Böventer-Heidenhain & Lowry, 1957) has shown these cations to be arranged in depth on the cell surface rather than in a single layer. A different experimental approach, that of determining the zeta potential of bacterial cells, has also shown the interaction of metal cations and cell components to occur at the external cell surface (Davies, Haydon & Rideal, 1956).

This proposed mode of action of metal cations has been criticized by Miller (1960) to whom reply has been made elsewhere (Somers, 1960). It should be emphasized that although the primary fungistatic action of the cations is regarded as due to non-specific reaction outside the cell protoplast, ultimate fungicidal action may well be caused by secondary reactions following the formation of un-ionized complexes. Mechanisms visualized could include the breakdown of membrane permeability and the diffusion of the metal ion complex into the interior of the cells.

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Concentrate spraying of apple trees

II. Dosage/volume relationships of lime-sulphur in disease control and spray damage

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SUMMARY

Following earlier indications of the importance of the quantity of lime-sulphur concentrate applied per acre (dosage) in the control of apple scab and powdery mildew, further intensive paint-gun experiments were designed to clarify basic dosage/volume relationships. They established that control depends primarily on the dosage and not merely on the volume of spray applied, but the volume was in some circumstances a critical factor in spray damage.

Incidental data showed the influence of lime-sulphur dosage also on the population of fruit tree red spider mite and on infestations of sawfly. There was evidence that the incidence of fruit russet and poor skin finish is sometimes related to impaired foliage.

Gravity flow and droplet-size of spray together increased with increasing dilution of lime-sulphur, and thus influenced spray distribution on the foliage when a range of dilutions was applied at fixed volume/acre.

INTRODUCTION

Intensive experiments had shown that air-blast applications of lime-sulphur (LS) concentrate in small, discrete droplets controlled apple scab (*Venturia inaequalis* (Cooke) Wint.) and powdery mildew (*Podosphaera leucotricha* (Ell. & Everh.) Salm.) as effectively as high-volume sprays of dilute LS of approximately similar quantity of chemical per acre (Moore, 1957). When a suitable method of application had been found, the concentrate proved no more damaging to apple foliage than the high-volume spray applied at a lower quantity of chemical per acre.

Disease control depended on good spray cover and on maintaining the quantity of chemical per acre above an undefined limit, which apparently approximated to 3 gal. LS/acre for bush Cox's Orange Pippin trees about 6 ft. high on M. IX rootstock and planted at 12 ft. square.

In the present paper the term *dosage* is retained for the quantity of chemical applied per acre, whether neat or diluted with water, and the gallonage per acre—of whatever concentration—will be referred to as the *volume*. The experiments were designed to evaluate LS dosage biologically in terms of volume and concentration of spray-fluid applied. At fixed concentration, dosage increased with volume; conversely, at constant volume, dosage increased with concentration.

EXPERIMENTS, 1952–5

Materials and experimental methods

The trees and methods of spraying were those used in the previous experiments (Moore, 1957). Small-volume sprays were applied from a paint spray-gun with gravity feed and air-blast nozzle, powered by a 2½ h.p. mobile compressor. Dilute high-volume sprays were applied to run-off, usually from a mobile 2 h.p. unit supplying twin nozzles on one hand-lance, but occasionally from a larger outfit. Spray programmes were modified for seasonal conditions to give only partial control, as complete elimination of disease would have prevented comparisons.

In every experiment each tree sprayed by the paint-gun received a measured quantity, slightly adjusted where necessary to accommodate variability in tree-size. The basic quantity at 3 gal./acre was 45 ml./tree (302 trees/acre at 12 ft. square).

In 1952 the treatments were designed in three-tree randomized plots replicated five times, and thereafter were re-randomized each year in single-tree plots replicated nine times. A light fabric screen prevented spray-drift, and insecticidal sprays were applied separately as necessary.

Lime-sulphur treatments

1952. *Comparison of volumes per acre at maximum concentration.* Undiluted LS sprays at 1, 2, 4, and 6 gal./acre were compared with dilute sprays, applied hydraulically to run-off, at green cluster 2½%, pink-bud 2%, and petal-fall 1%. Three applications, two pre- and one post-blossom; completed 12 May.

1953. *Comparison of a wide range of dilutions at constant volume.* LS sprays at 3 gal./acre, in a geometric series of dilutions, viz. 1, 3, 9, 27, 81%, were compared with 1% sprays pre- and post-blossom applied to run-off by the paint-gun adjusted to a larger output. Four applications, two pre- and two post-blossom; completed 8 June.

1954. *Comparison of a limited range of dilutions at constant volume.* LS sprays at 3 gal./acre, at 25, 50, 75 and 100%, were compared with unsprayed controls and with high-volume sprays at the 1952 dilutions; as in 1953, however, another 1% spray was added after petal-fall. Four applications, two pre- and two post-blossom; completed 4 June.

1955. *Evaluation of constant dosage at variable volumes and dilutions.* LS sprays were compared at constant dosage, 3 gal. LS concentrate/acre, with unsprayed controls and high-volume sprays as in 1954, as follows: 100% at 3 gal./acre; 50% at 6 gal./acre; 25% at 12 gal./acre; 12½% at 24 gal./acre. Four applications, two pre- and two post-blossom; completed 6 June.

This final experiment provided a link between concentrate and medium-volume spraying, which was by then a common commercial practice at about 50 gal./acre. For orchard trees this volume would be roughly comparable to the 24 gal./acre used in the experiment.

Records

Estimations by category. The use of category values as a means of comparing degrees of infection or spray damage (Moore, 1930) is especially suitable for small trees on which most of the foliage can be seen from above, and where these rapid interim assessments can be linked with more detailed records. Conformity of assessments by individual observers can be safeguarded by training, experience, and clear definition of categories after the general range of infection etc. has been surveyed. Quick assessment is desirable and should not be sacrificed to tedious counting, which may prove no more informative; moreover, subdivision into numerous splinter-categories often confuses rather than clarifies the recording.

Category records are best arranged stepwise on an approximate numerical basis; e.g. with five disease categories (1–5) and one freedom category (nil), cat. 1 might include every tree with up to five or ten infected leaves (about 0·05–0·1 % on these small bush Cox's). The remaining steps would be based on the range of infection, with a constant factor or interval appropriate to the biological increment. For more static biological phenomena (e.g. spray scorch) five steps each of 20 % may be more convenient; thus cat. 1, 1–20 % leaves affected; cat. 5, 80–100 %.

The category values in the tables are means/tree (including nil) of such assessments; the definitions used in each instance are omitted, but maxima are shown as a measure of relative severity.

The severity index. This index was used for estimating scab on spur leaves, and scab and russeting on picked fruits. Appropriate random samples were graded according to surface area of scab or russeting, and the severity index was computed as percentage × mean grade (Moore, 1957). Where grading was difficult, as with mildew on spur leaves, or unnecessary, as with sawfly on dropped fruitlets, the values were expressed as a simple percentage.

Fruit tree red spider mite (Metatetranychus ulmi Koch).* A sample of eight random leaves of similar age from each tree was recorded by the imprint method (Austin & Massee, 1947).

Crop. The number of fruits set or mature/100 blossom trusses was computed from counts of 200 blossom trusses marked off on sample branches of each tree.

Dullness of fruit 'finish'. This value was assessed from inspection of the fruit in trays as picked.

Assessment of chemical deposits. Estimates of the amount of LS retained on leaf surfaces as a result of the treatments were made on six of the blocks in 1953–4–5, post-blossom only, by the following method. Each block provided one tree per spray treatment and (except in 1953) one control tree. Before each spray application four leaves were removed from every control tree, one from each quarter, and four similarly from every sprayed tree immediately after treatment. In 1953, control leaves were obtained by removing one leaf from each tree in a block before any trees were sprayed.

Every leaf was individually immersed in 10 ml. of buffer solution, and then stored on a wire; the amount of LS thus washed into the buffer solution was later estimated by

* 'Red spider' in subsequent text.

the method of Allen & Llewelyn (1954), and meantime the leaves were dried overnight at 80° C. in the laboratory and then weighed. The volume of LS (expressed as 100% LS) retained per gram dry weight of leaf was calculated for each individual leaf, and the mean value for four leaves per tree computed.

Transformation of data for analysis. Where transformations were made, the original data or their re-transformed values are shown, with the transformed values and their significant differences in italics beneath. Percentages were transformed to angles of equal information, red-spider counts to log $(n+\frac{3}{8})$, and some severity indexes (in 1955) to square roots. Appropriate correction was also made for any effect of previous treatments.

RESULTS

The results are classified in the following tables as mean values/tree.

1952. *Comparison of volumes of LS/acre at maximum concentration*

Disease control and spray damage to foliage. The concentrate sprays show two distinct pairs of affinities: (*a*) 1 and 2 gal./acre and (*b*) 4 and 6 gal./acre. Neither 1 nor 2 gal. was sufficient to control scab and mildew well, and neither caused any damage of consequence; 4 and 6 gal., however, each gave good control but caused fairly severe damage, especially 6 gal., which, deposited partly as a film, was evidently far more than trees of this size could tolerate (Table 1).

The high-volume treatment gave good control, broadly consistent for a comparable dosage (mean 3·3 gal./acre) interpolated in the concentrate range, but the pre-blossom sprays (to 30 April; mean, 4·05 gal./acre) caused much more leaf-scorch (3·87) than the concentrate spray (2·40) of similar dosage.

Crop data. Although these data reflect the foliage damage, inconsistencies suggest the influence of other factors also; for instance, there was a greater crop on trees sprayed at 6 than at 4 gal./acre. One of these factors was probably sawfly attack, reduced as the volume and dosage of LS increased, although nicotine (0·05%) and spreader had been hydraulically applied separately at petal-fall for its control. Differences were not due to floral injury, and other indications of the bearing of this fungicide on sawfly control are recalled (Moore, 1933; Dicker & Tew, 1957). The greatest crop was on trees sprayed at 2 gal./acre.

Red spider. There was a remarkable increase in the red-spider population in June where the spray concentrate had been applied at 2 gal./acre, but detailed records of the predator status of individual plots would be necessary to explain it fully. It seems likely that, at this dosage, the spray may have killed *Typhlodromus* sp. early in the season (Collyer & Kirby, 1959) and thus caused most disturbance to the pest/predator equilibrium, whereas at the higher dosages, including that of the run-off spray applied pre-blossom, the sprays were lethal to pest and predator alike. At petal-fall, however, the dosage of the run-off spray was reduced to 1·8 gal./acre, and, by July, trees so sprayed had developed as high a population of red spider as that associated with the concentrate at similar dosage (2 gal./acre)—a further indication of the failure of predatory activity to take over red-spider control when spraying ceased. The only instance in which the total red-spider population showed no increase from 11 June

Table 1. *Disease and pest control, and incidence of spray damage*, 1952

Three sprays; two pre- and one post-blossom (final, 12 May).

	Small-volume sprays				Run-off spray	Significant difference		
Volume (gal./acre) ...	1	2	4	6	180			
LS at ...	100 %	100 %	100 %	100 %	(2½ %, 2 %; 1 %)			
Dosage (gal. LS/acre) ...	1	2	4	6	(4·5, 3·6; 1·8)			
Records					(mean, 3·3)	5 %	1 %	0·1 %
				Disease and pest control				
Scab on foliage (cat. max. 5) 26 June	1·63	1·47	0·70	0·37	0·67	0·34	0·47	0·64
Scab on picked fruit (sev.)	19·6	21·3	2·6	2·9	3·4	6·7	9·3	12·8
Mildew on spur leaves (%)	5·1	2·6	1·4	0·7	1·4	—	—	—
2 July	*13·0*	*9·2*	*6·6*	*4·9*	*6·7*	*2·4*	*3·4*	*4·6*
Sawfly on dropped fruitlets (%)	26·4	15·3	11·6	4·8	12·9	—	—	—
July	*30·9*	*23·0*	*19·9*	*12·6*	*21·0*	*7·9*	*10·9*	*15·1*
Red spider on foliage								
11 June								
No. mites	18	60	11	23	17	—	—	—
	1·26	*1·78*	*1·07*	*1·38*	*1·24*	*0·28*	*0·39*	*0·53*
No. eggs	507	1057	219	352	415	—	—	—
	2·71	*3·02*	*2·34*	*2·55*	*2·62*	*0·28*	*0·39*	*0·53*
16 July								
No. mites	37	108	46	46	130	—	—	—
	1·57	*2·04*	*1·67*	*1·67*	*2·12*	*0·34*	*0·47*	*0·65*
No. eggs	486	1272	530	641	1266	—	—	—
	2·69	*3·10*	*2·72*	*2·81*	*3·10*	*0·20*	*0·28*	*0·39*
				Spray damage				
Leaf-scorch (cat. max. 5) 30 April	0·33	0·87	2·40	3·20	3·87	0·42	0·58	0·79
Leaf-scorch (cat. max. 5) 21 May	0·80	0·73	1·93	2·60	1·00	0·71	0·98	1·34
Leaf-drop (cat. max. 5) 21 May	1·60	1·20	2·00	2·33	1·07	0·44	0·60	0·83
No. fruit set/100 blossom trusses	110·4	162·6	88·7	106·0	161·2	34·0	46·9	64·4
Mature crop: no. fruits/tree	98·8	233·5	69·0	112·0	140·2	50·1	69·3	95·7

Table 2. *Assessment of chemical deposits, disease and pest control, and cropping,* 1953

Four sprays; two pre- and two post-blossom (final, 8 June).

	Small-volume sprays					Run-off spray*	Significant difference		
Volume (gal./acre) ...	3	3	3	3	3	36			
LS at ...	1%	3%	9%	27%	81%	1%			
Dosage (gal. LS/acre) ...	0·03	0·09	0·27	0·81	2·43	0·36	5%	1%	0·1%
Records									
Assessment of chemical deposits									
(ml. 100% LS/g. dry wt. of leaf)									
May	0·007	0·006	0·009	0·017	0·034	0·015†	0·012	0·016	0·022
June	0·010	0·010	0·012	0·025	0·039	0·016†	0·008	0·011	0·015
Mean	0·009	0·008	0·011	0·021	0·037	0·016	—	—	—
Disease and pest control									
Scab on foliage (cat. max. 5) 22 June	1·9	2·3	1·2	1·0	0·8	1·1	0·5	0·7	0·9
Scab on spur leaves (sev.) 22 Aug.	18·1	11·0	10·5	7·9	3·9	8·6	5·1	6·8	9·0
Scab on picked fruit (sev.)	54·6	60·3	28·1	22·1	9·1	12·2	23·0	30·7	40·4
Mildew on spur leaves (%) 2 Sept.	32·3	28·8	22·6	15·6	5·5	18·1	—	—	—
	34·6	*32·4*	*28·4*	*23·3*	*13·6*	*25·2*	*4·8*	*6·4*	*8·4*
Red spider on foliage (mites and eggs)									
22 June	562	501	380	302	52	281	—	—	—
	2·75	*2·70*	*2·58*	*2·48*	*1·72*	*2·45*	*0·25*	*0·33*	*0·44*
16 July‡	1949	1479	1548	1258	501	1023	—	—	—
	3·29	*3·17*	*3·19*	*3·10*	*2·70*	*3·01*	*0·21*	*0·27*	*0·36*
Leaf-bronzing (cat. max. 5) 23 July	2·8	2·8	2·3	2·3	1·1	1·7	0·7	0·9	1·2
Crop records									
No. fruit set/100 blossom trusses	145·2	157·2	188·5	167·1	130·0	151·6	N.S.	—	—
No. mature fruit/100 blossom trusses	24·7	31·8	41·6	37·3	29·4	44·2	13·4	17·9	23·6
Mature crop: no. fruits/tree	140·2	187·2	209·9	181·0	145·9	138·7	N.S.	—	—
Russet on picked fruit (sev.)	65·3	51·6	49·3	40·0	36·9	35·4	15·3	20·5	26·9
Dullness of fruit finish (cat. max. 4)	2·3	2·2	2·2	1·7	1·6	2·1	0·5	0·6	0·8

* Applied by paint-gun.

† For unsprayed trees the value was 0·004 in both May and June.

‡ By 16 Aug. there were no significant differences, but note that the final spray was on 8 June.

to 16 July was on trees concentrate-sprayed at 1 gal./acre, so there was evidently an adequate reserve of predatory activity following this otherwise unsatisfactory treatment.

Conclusions, 1952

Within certain limits both spray damage and disease and pest control increased with increasing dosage. At 2 gal./acre or less the concentrate spray was not damaging but was insufficient for good disease control, while at 4 gal./acre or more it was effective but caused much leaf-scorch, especially at 4 gal./acre as a dilute spray.

The main cause of spray damage seemed to be the application of high dosage in a volume of spray too great for deposition as small, discrete droplets. The optimum volume of LS concentrate for these trees evidently lay between 2 and 4 gal./acre, but the influence of the quantity of chemical acting independently of the volume of spray was not yet clear. In 1953–4, therefore, a range of dilutions at constant volume (3 gal./acre) was investigated. The results are assembled in Tables 2 and 3.

1953–4. *Comparison of LS dilutions at constant volume/acre*

Disease control. Apart from minor fluctuations, control improved linearly with dosage of LS, whether the spray was applied as discrete droplets at 3 gal./acre or to run-off by paint-gun. The lowest dosages provided negligible deposits and failed to control disease, but probably owing to inferior cover as well as reduced dosage. Measured volumes of the weaker sprays were used up more quickly than those of the 81 % dilution, and demanded greater speed in covering the entire tree. The following tests* showed that the rate of unregulated gravity flow of LS, and therefore the droplet size, increased with dilution (owing to decreasing viscosity), thereby decreasing the number of droplets per unit volume of spray.

Concentration of LS (%)	100	75	50	25	$12\frac{1}{2}$	$6\frac{1}{4}$	0 (water)
Flow rate (ml./min.)	39	63	91	111	125	124	129
Mass median diam. of droplets (μ)	33	42	45	58	63	63	—

These values support earlier field observations that a greater volume of 50 % than of 100 % LS was required to provide adequate cover, and, with gravity feed, a dosage deficiency of the diluted spray thus tends to be automatically compensated (Moore, 1957). Mean diameter (63 μ) of droplets at $12\frac{1}{2}$ % was nearly double that (33 μ) at 100 %, so there would be nearly 2^3 more droplets at 100 % from the same volume of spray, though their impaction potential is lower. To improve the cover and to increase the dosage deposited by the weaker sprays, increases in volume per acre would be necessary. The 1 % spray intensively applied by paint-gun to run-off at 36 gal./acre is a good example, and evidently corresponds in volume, though, at its reduced dosage, not in performance, to 180 gal./acre (cf. 1952) applied as a hydraulic spray.

It can be concluded that sprays of low concentration need relatively high-volume application if only to ensure a sufficient quantity of LS for satisfactory disease control.

Red spider. The results show a similar pattern of dosage response to those for scab

* The tests were made in collaboration with J. B. Byass of the N.I.A.E., with the paint-gun unit as operated in the field experiments.

and mildew, although in this case the run-off spray consistently gave slightly better control for equivalent dosage, possibly owing to a mechanical effect of its greater volume. Only the highest dosage (2·43 gal.) however, appreciably reduced the spider population. The degree of leaf-bronzing caused by the attack closely followed the population counts; the green colour of the foliage on the randomized single trees with 81 % LS provided a marked contrast about 6 weeks after the final spray on 8 June, though by then clearly beginning to show the effect of mites that had migrated from the neighbouring heavily infested trees.

Crop data. Trees varied widely in the amount of harvested crop, which was highest on those receiving the 9% spray, i.e. 0·27 gal. LS/acre. This non-significant pattern was reflected also by the fruit/blossom-truss ratios based on branch sampling; a significant difference in favour of the run-off spray was inconsistent.

The clear negative correlation at 3 gal./acre between spray dosage and fruit russet/skin 'finish' is arresting because the reverse might have been expected from experience of high-volume LS sprays, which sometimes cause russeting. Better pest and disease control at the higher dosage greatly improved the foliage, which evidently protected the fruits from cold wind, sunburn, excessive transpiration, and other causes of skin deterioration (Moore & Bennett, 1952). The 1 % spray applied to run-off, like the 81 % concentrate, produced good foliage and least fruit russet, but the fruit 'finish' was impaired (cat. 2·1) by red spider.

Conclusions, 1953

Disease control depended primarily on good cover coupled with adequate spray dosage, and was then largely independent of volume. As in 1952, dosages of less than 2 gal. LS/acre were unsatisfactory. In 1954, a closer range of dosages (mean *c.* 2 gal. LS/acre) at 0·75 gal. intervals was therefore investigated to determine optimum dosage, again at a constant volume of 3 gal./acre. The results are shown in Table 3.

Conclusions, 1954

All treatments reduced both scab and mildew, and a response to dosage was again evident at high infection levels. Neither scab nor new mildew infection occurred pre-blossom, as the dry, cold weather was unfavourable, so the greater dosage of the pre-blossom high-volume sprays probably contributed little to their fungicidal value (Moore, Kirby & Bennett, 1961).

Dosages below 2 gal./acre were again inferior, but 3 gal./acre was in no way superior to 2·25 gal. As it was equally safe, however, 3 gal. would clearly be the more reliable under epidemic conditions and with less intensive application. It was therefore chosen as optimum dosage for these trees in a final experiment to test the hypothesis that, given adequate cover, the fungicidal effectiveness of optimum dosage does not depend critically on the volume of spray applied, although volume may influence the degree of spray damage. The results are shown in Table 4.

Table 3. *Assessment of chemical deposits, disease control, and cropping*, 1954

Four sprays; two pre- and two post-blossom (final, 4 June).

	Small-volume sprays				Run-off spray		Significant difference		
Volume (gal./acre) ...	3	3	3	3	180				
LS at ...	25 %	50 %	75 %	100 %	(2½ %, 2½ %; 1 %, 1 %)				
Dosage (gal. LS/acre) ...	0·75	1·50	2·25	3·00	(4·5, 3·6; 1·8, 1·8) (mean, 2·9)	Unsprayed controls	5 %	1 %	0·1 %
Records									
Assessment of chemical deposits									
(ml. 100 % LS/g. dry wt. of leaf)									
May	0·022	0·035	0·042	0·067	0·035	0·010	0·019	0·026	0·034
June	0·017	0·017	0·025	0·031	0·018	0·007	0·007	0·010	0·014
Mean	0·020	0·026	0·034	0·049	0·027	0·009	—	—	—
Disease control									
Scab on foliage (cat. max. 5) 19 July	1·3	1·3	0·7	1·2	0·9	2·4	0·7	0·9	1·2
Scab on spur leaves (sev.) 11 Aug.	3·1	2·6	1·0	1·4	0·8	8·7	3·2	4·3	5·7
Scab on picked fruit (sev.)	30·7	27·1	11·4	5·9	9·9	68·3	22·1	29·7	39·2
Mildew on foliage (cat. max. 5) 4 Aug.	2·1	2·3	1·8	1·9	1·9	2·5	0·4	0·6	0·7
Mildew on spur leaves (%) 13 Aug.	22·0	20·2	12·1	9·0	15·5	58·1	—	—	—
	27·9	*26·6*	*19·6*	*16·1*	*22·6*	*49·4*	*6·1*	*8·2*	*10·8*
No. mildewed buds/tree Apr. 1955	2·6	1·0	0·3	1·0	0·8	6·1	2·9	3·9	5·1
Crop records									
No. mature fruit/100 blossom trusses	30·1	42·9	45·0	47·0	35·4	24·5	15·5	20·8	27·4

There were no significant differences for no. of picked fruit per tree, fruit russet, or red spider. Re-randomization of treatments every year redistributes any residual red-spider pattern.

Table 4. *Assessment of chemical deposits, disease control, and incidence of spray damage*, 1955

Four sprays; two pre- and two post-blossom (final, 6 June).

	Small-volume sprays				Run-off spray		Significant difference		
Volume (gal./acre) ...	24	12	6	3	180				
LS at ...	12½ %	25 %	50 %	100 %	(2½ %, 2 %; 1 %, 1 %)				
Dosage (gal. LS/acre) ...	3	3	3	3	(4·5, 3·6; 1·8, 1·8) (mean, 2·9)	Unsprayed controls	5 %	1 %	0·1 %
Records									
Assessment of chemical deposits									
(ml. 100 % LS/g. dry wt. of leaf)									
May	0·030	0·053	0·035	0·026	0·015	0·003	0·015	0·021	0·028
June	0·032	0·032	0·021	0·029	0·017	0·003	0·010	0·014	0·019
Mean	0·031	0·043	0·028	0·028	0·016	0·003	—	—	—
Disease control									
Scab on spur leaves (sev.) 12 Aug.	0·25	1·00	0·49	0·04	0·25	24·01	—	—	—
	0·5	*1·0*	*0·7*	*0·2*	*0·5*	*4·9*	*0·7*	*0·9*	*1·2*
Scab on picked fruit (sev.)	2·25	4·00	3·61	2·25	1·44	88·36	—	—	—
	1·5	*2·0*	*1·9*	*1·5*	*1·2*	*9·4*	*1·6*	*2·2*	*2·9*
Mildew on spur leaves (%) 13 July	16·4	13·6	14·2	12·0	12·3	45·0	—	—	—
	23·9	*21·6*	*22·1*	*20·3*	*20·5*	*42·1*	*6·2*	*8·4*	*11·1*
Mildew on foliage (cat. max. 5) 27 July	1·9	2·0	2·1	1·5	1·8	2·6	0·4	0·6	0·8
Spray damage									
Leaf-scorch (cat. max. 4) 27 April	2·3	1·3	1·0	0·9	2·4	0·0	0·5	0·7	0·9
Leaf-scorch (cat. max. 4) 2 June	2·5	1·4	1·5	1·2	1·2	0·0*	0·4	0·5	0·6
Russet on picked fruit (sev.)	65·8	68·3	91·9	67·1	77·7	106·2	24·5	32·9	43·4
Dullness of fruit finish (cat. max. 4)	1·9	1·9	2·0	1·7	2·1	2·3	0·4	0·5	0·6

There were no significant differences for crop or red spider.

* 'Cox blotch' developed in late June.

1955. *Evaluation of constant LS dosage at variable volumes/acre and dilutions*

Disease control. All treatments gave a high degree of scab control in a season of very heavy infection, i.e. control clearly depended more upon the quantity of chemical applied than upon the volume of the spray. Further, the high-volume spray gave better control on the fruit than in 1954, probably because the higher quantity of LS in the pre-blossom applications played a greater part in 1955. The only significant difference between the small-volume sprays (that relative to scab on spur leaves) favoured the undiluted concentrate. Mildew control was failing some 6 weeks after the final spray, but the only significant difference also favoured neat LS. Deposition was consistent with dosage applied except at 25% LS in May and at 50% in June; these are unaccountable but significant variations, although they were not reflected by the biological data.

Spray damage. Leaf-scorch category 1·0 represents very slight damage that might well escape notice in commercial practice; 2·0 or more, however, would cause concern. The sharp distinction in this respect between the 12- and 24-gal. volumes is therefore of considerable practical importance, and indicates the change from a concentrate type of spray to one more akin to a medium- or high-volume spray (and see flow rates, p. 260), in which the droplets tend to merge into a film, as on these trees. The pre-blossom high-volume spray caused a similar degree of damage, but, as the concentration was reduced post-blossom, leaf-scorch (June) was practically negligible, though still moderate to severe from the 24-gal. spray of nearly double the dosage.

By the end of June the control trees especially showed leaf-scorch of a different kind; it was brown, blotchy, and chiefly interveinal, not marginal like much of that associated with the sprays. This blotching of Cox leaves is sometimes common and cannot always be traced to spray treatments; indeed, in the present instance, records showed that there was twice as much on unsprayed trees (mean cat. 2·1) as on the sprayed (mean cat. 1·0). It seems therefore that LS in some way mitigates this malady, which may be nutritional.

The unsprayed fruits were most russeted (Moore, 1932), which strongly endorses the evidence that poor foliage was largely responsible (p. 261). Among the sprays, only the 50% concentrate significantly increased russeting above the general level, although the data for deposits show no evidence of over-application of this spray. Russeting was not increased on trees that earlier showed most marginal leaf-scorch. Neat LS yielded the fruits with the brightest skin (cat. 1·7).

DISCUSSION AND GENERAL CONCLUSIONS

These experiments have substantiated earlier indications (Moore, 1957) that the quantity of chemical applied per acre (dosage), whether as a low-volume or a high-volume spray, is a basic factor in the control of apple scab and apple mildew by LS. There was additional evidence that the control of red spider and of apple sawfly also was influenced by the dosage of LS applied. Later field experience has shown that some other fungicides have proved effective against scab and mildew as low-volume sprays where the dosage has not been allowed to fall below the quantity usually effective when applied by high-volume methods.

Most of the values for chemical deposition reflected the range of dosage applied, and were consistent with the biological performance of the treatments. The few minor though sometimes significant inconsistencies made no apparent difference to the biological effectiveness of any sequence of sprays as a whole.

For these small trees the optimum LS dosage was 2·25–3 gal./acre, but the higher rate (= 7·5 lb. soluble sulphur) would be the more reliable where, as in automatic spraying, the spaces as well as the trees are sprayed, and also in epidemic conditions. Established trees of medium size would probably require at least 4–5 gal. LS/acre to ensure similar cover and disease control, i.e. 10–12·5 lb. sulphur (Moore, 1958). In terms of high-volume spraying, this dosage is the equivalent of 200–250 gal./acre of 2% LS or of about 300 gal. of 1·5%; these are usual pre-blossom applications, which, particularly when made by hand, are highly effective but may cause leaf-scorch, especially in cold wind. When reduced to 1% or less to obviate damage, however, LS may sometimes fail to give adequate control of scab, mildew, or red spider in epidemic conditions through lack of sufficient chemical on the young leaves and fruits at the critical time. In these circumstances the degree of control will obviously depend on whether or not the main scab-infection periods, arising from ascospores, occur before or after the reduction to 1% is made. Application pre- and post-blossom of LS at appropriate dosage as a concentrate (100–25% according to these experiments, but especially 100%) would counter this difficulty without the risk of severe damage. There was no evidence in these experiments of crop reduction by the small-volume concentrates finally selected, but in practice careful application is necessary by a suitable machine delivering the correct type of spray (Moore, 1958).

Given optimum dosage and satisfactory cover as small, discrete droplets, increases in spray volume made little difference to disease control, but caused leaf-scorch when the droplets merged into a film and dried more slowly. This point was reached at 24 gal./acre of the 1 in 8 dilution (12½% LS) in these experiments, but about double this volume would probably be required for similar cover on trees of more usual commercial size. Some 50 gal./acre of 12½% or even 10% LS is, however, much too phytotoxic to be practicable (Moore, 1958). Optimum dosage is therefore least damaging at small volumes, but medium-volume sprays could be made safer by reducing the concentration, and therefore the dosage, to one-half or less. Although *a priori*, this device will probably reduce effectiveness, as with high-volume sprays, it might be compensated by more frequent spraying unless this alternative also is ruled out by phytotoxicity.

The crop from trees on which scab, mildew, and red spider were not well controlled showed increased fruit russeting, probably from reduced protection against adverse weather conditions by the impaired foliage. Spray treatments themselves, however, sometimes cause russeting (e.g. 50% LS in 1955).

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Effect of time and temperature on toxicity of insecticides to insects

III. Tests of seven poisons in the range 10–28° C.

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SUMMARY

The contact poisons rotenone, 5,5-dimethyldihydroresorcinol dimethylcarbamate ('Dimetan'), 2-bromomercurithiophen, 2-*iso*valeryl-1,3-indandione ('Valone'), α-chlordane, toxaphene and DDT were tested, in probit assays, on as many as possible of four insect species (*Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Tenebrio molitor* L., and *Musca domestica* L.) by applying the poison so that there was no 'pick-up' effect, and then keeping the insects at each of two post-treatment temperatures (usually 10 and 28° C.) for as long as possible.

The same insects were counted repeatedly throughout each test. At each counting, the two ED 50's were found and from these the temperature coefficient of toxic action (ratio of ED 50's) was calculated; temperature coefficients were 'positive' or 'negative', according to whether the toxic action was greater or less at the higher temperature. The time for each ED 50 to decrease to a steady value (the end-point) was also found; the inverse of this time was the 'speed of action' of the poison. In some tests the end-points were not reached, even though the insects were kept until the proportion dead in the control batches reached about 40%. Temperature coefficients, measured soon after treatment, were most probably temperature coefficients of paralysis only; and those at the end of the test, coefficients of kill, with continuous gradation between.

The results were characteristic of the poison used, and not of the test species.

Rotenone and 'Dimetan' each caused an initial paralysis, from which the insects temporarily recovered before dying. With rotenone, increase in post-treatment temperature increased the speed of the sequence knockdown–recovery–death, and probably the speed of action; the initial temperature coefficient (of paralysis) was negative, but it changed to a positive coefficient as time passed; end-points were not reached. Results with 'Dimetan' were somewhat similar, but the coefficients were very small and variable in sign.

With 2-bromomercurithiophen, 'Valone', α-chlordane and toxaphene, the transition from the initial paralysis to death was not interrupted by a period of recovery; all the ED 50's decreased steadily in size as time passed, and the observed decrease was greater at the lower temperature. Increase in post-treatment temperature nearly always increased the speed of action, which was greater with 'Valone' than with any

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of the other poisons. The temperature coefficients were initially positive, but became smaller or sometimes negative as time passed, so that an increase in post-treatment temperature either did not affect the ultimate toxicity (2-bromomercurithiophen and some tests with 'Valone') or decreased it (α-chlordane, toxaphene and the other tests with 'Valone'); increase in post-treatment temperature also increased the curvature of the line relating ED 50 to time after treatment.

In the tests with DDT, the ED 50's also decreased steadily as time passed, but the temperature coefficients were consistently negative. Increase in post-treatment temperature did not affect the shape of the ED 50–time curve; no general statement can be made about the effect of temperature on speed of action.

Thus, a change in post-treatment temperature can affect the course of poisoning of contact insecticides by affecting their speed of action (which usually increased with post-treatment temperature), or the ultimate toxicity, or the shape of the ED 50–time curve, or in some combination of these ways.

Some of the explanations of the negative post-treatment temperature coefficient of DDT are discussed.

INTRODUCTION

Some of the terms convenient for describing the effects that temperature may have on toxicity of insecticides to insects were defined by Pradhan & Mundkur (1957) and Das & Needham (1961); others are now defined.

In laboratory tests of contact poisons, in which measured doses are given to the insects, it is commonly although not always found that the percentage affected by any one dose increases as time passes after the dose is applied, finally reaching a steady value. The percentage affected is usually found by adding together the percentages paralysed and dead. Within this group, the ratio of dead : paralysed insects also usually increases as time passes; near the beginning of a test, most of the 'affected' insects are only paralysed; at the end, all of them are dead. In practice, it is convenient to follow the decrease in ED 50, or dose needed to affect 50% of the insects (Finney, 1952), to a steady value (cf. Bettini, Boccacci & Natalizi, 1958; Das, 1961), although the ED 50 is no measure of the ratio of dead : paralysed insects. The time at which the steady value is reached is the end-point of the test (Beard, 1949) and, in this paper, the ED 50 at the end-point is called the 'end-point ED 50'.

'Speed of action' has been used to mean the inverse of the time taken for a poison to bring about a certain effect, like 50% paralysis (cf. Häfliger, 1954); however, this definition is unsatisfactory, because different doses have different 'speeds of action'. In this paper, 'speed of action' means the inverse of the time taken for the end-point to be reached; by this definition, in our experience, different doses have the same 'speed of action'.

However, the end-point ED 50 and the speed of action are not themselves enough to describe the course of events between dosing and the end-point, because the 'shape of the curve' relating ED 50 to time may vary from one set of circumstances to another.

The general effects that changes in temperature may have on toxicity of insecticides are well known. The aim of this paper is to show whether changes in post-treatment

temperature change the end-point ED 50, or the speed of action, or the shape of the curve, or any combination of these three.

To this end, seven contact poisons of various chemical types were tested on as many as possible of four insect species. The poisons were heat stable, and had little or no fumigant action. Doses were applied to the insects by methods in which there were no 'pick-up' effects (Pradhan, 1949; Pradhan & Srivastava, 1956). Afterwards the insects were kept at each of two temperatures, and the same insects were counted repeatedly at intervals over as long a time as possible. The ratio of the ED 50's at the two temperatures is taken as the ' temperature coefficient ' of toxic action, and the temperature coefficient which persists after both end-points have been reached is the 'end-point temperature coefficient'; numerical values for temperature coefficients are preceded by + or −, according to whether the toxic action was greater or less at the higher temperature.

MATERIALS

Insecticides

Rotenone, m.p. 161–163° C.

5,5-Dimethyldihydroresorcinol dimethylcarbamate ('Dimetan'), m.p. 41° C.; from the Geigy Co., Basle.

2-Bromomercurithiophen, m.p. 164–169° C.; prepared by the method of Volhard (1892) and Steinkopf (1917).

2-*iso*Valeryl-1,3-indandione ('Valone'), m.p. 65·5–66° C.; prepared by the method of Kilgore, Ford & Wolfe (1942).

α-Chlordane, m.p. 102–104° C.; from Velsicol Corp., Chicago, Ill.

Toxaphene, technical; from Hercules Powder Co., Wilmington, Del.

DDT, m.p. 106–107° C.

Adjuvants

Sulphonated Lorol (containing about 55% technical sodium lauryl sulphate); from Ronsheim & Moore.

White saponin; from Hopkin & Williams.

Insect species

Saw-toothed grain beetles, *Oryzaephilus surinamensis* (L.), reared at 30° C. on rolled oats. Adults were used, from 2- to 3-month-old cultures.

Rust-red flour beetles, *Tribolium castaneum* (Herbst), reared at 28° C. on wholemeal flour. Adults were used from 2-month-old cultures.

Mealworms, *Tenebrio molitor* L., reared at 20° C. on bran and potatoes. Adults were used, about 2 weeks after emergence.

House flies, *Musca domestica* L., of a normally susceptible strain, reared at 28° C. by the method of Sawicki & Thain (1961). Adult females were used, 5–7 days after emergence.

METHODS

Formulation

Dipping. Colloidal suspensions of rotenone, 2-bromomercurithiophen and DDT, and suspensions of crystals of 2-bromomercurithiophen, were made by the methods of McIntosh (1957*b*); 'Dimetan' was dissolved in water containing 0·1% Sulphonated Lorol.

Topical application. The poisons were dissolved in toluene (for *T. castaneum*), rectified acetone (for *T. molitor*) or highly purified ('Analar') acetone (for *M. domestica*).

All solutions and colloidal suspensions were used when freshly made; the suspensions of crystals of 2-bromomercurithiophen were used the day after preparation.

Tests of fumigant action

These were at 10° and 28° C.

The petri dish methods used for *O. surinamensis* and *T. castaneum* have been described by McIntosh (1954); and the method for *T. molitor*, also used now for *M. domestica*, by McIntosh (1957*b*). The *T. castaneum* were placed in 1½ in. tubes, closed by tightly stretched muslin, and not on the floors of the dishes. Food was given to *T. molitor* (sliced potato) and *M. domestica* (paste of sugar and dried milk). In the tests on *T. molitor* the dishes were opened daily to change the air. *M. domestica* could be kept for only 1 day in this kind of test.

Dipping tests on Oryzaephilus surinamensis

About 10 ml. of the solution or suspension was poured on to each batch of sixty or seventy adults in a 2 × 1 in. specimen tube, which was then stoppered with a rubber bung and shaken gently by hand at room temperature for 2 min. The insects were transferred, by the wire-mesh method (McIntosh, 1947) to clean dry specimen tubes, and these were closed by tightly stretched nylon parachute fabric. No food was added.

When the insects were to be kept at 100% relative humidity (R.H.), they were put, in 2 × 1 in. specimen tubes, in desiccators over $CaCl_2$ for the first 24 hr. after dosing, to dry the adhering water. After 24 hr. the tubes were placed on their sides in sealed 9 cm. Petri dish pairs, as in the tests of fumigant action (McIntosh, 1954); each Petri dish pair also contained a small open tube of water.

In each test, one batch of sixty or seventy insects was used for each concentration of poison at each post-treatment temperature. The insects were kept for up to 12 days at 10° C. and up to 4 days (uncontrolled humidity) or 7 days (100% R.H.) at 23 or 28° C.

Topical application tests on Tribolium castaneum

About ten CO_2-gassed adults were placed on a piece of filter-paper and, when just beginning to recover, were pushed on to their legs with a paint brush. A droplet of toluene solution (0·125 μl.) was applied to each through a blunt cannula (0·3 mm. external diam.) attached to a micrometer syringe. So far as possible, the droplets

were placed on the elytral suture of each insect, near the junction of the thorax and abdomen. No food was given.

When the insects were to be kept at uncontrolled humidity they were put, in batches of twenty, in open 2 × 1 in. specimen tubes, each containing a piece of folded filter-paper. When they were to be kept at 100% R.H. they were put, in batches of fifty or sixty, on the filter-paper covered floors of sealed 9 cm. Petri dish pairs as in the tests of fumigant action (McIntosh, 1954); each Petri dish pair also contained a small open tube of water.

In each test, three batches of twenty, or one of fifty or sixty were used for each concentration of poison at each post-treatment temperature. The insects were kept for up to 24 days (10 and 28° C.).

Topical application tests on Tenebrio molitor

The methods have been described by Das (1961). 4 μl. acetone droplets were topically applied in all tests except those with DDT (3 μl.). Sliced potato was put in the dishes at the start of each test, or on the 3rd day after treatment.

In each test, two batches of fifteen insects were used for each concentration of poison at each post-treatment temperature. The insects were kept for up to 24 days (10 and 28° C.).

Topical application tests on Musca domestica

Droplets (1 μl.) of acetone solutions were applied to flies by the method described by Sawicki & Thain (1961). After treatment, the flies were kept, in batches of twenty, in 9 cm. Petri dishes closed by tightly stretched muslin. Each dish contained some food (dried milk and sugar, 1:1) and some moist cotton wool, which was remoistened daily by water from a capillary tube pushed through the muslin.

In each test, two batches of twenty flies were used for each concentration at each post-treatment temperature. The insects were kept for up to 12 days (10° C.) or 5 days (28° C.).

General notes on testing and on presentation of results

All tests followed the general design of probit assays (Finney, 1952); in each, the effects of two post-treatment temperatures were compared.

All doses were applied at room temperature, and the insects were at once placed at their post-treatment temperature (10 and 28° C. or, sometimes, 23° C.). In each test, the same insects were counted repeatedly at intervals up to about 3 weeks after treatment. At each counting, one or two batches of insects were taken out of the constant-temperature cabinet or room, counted at room temperature as quickly as possible and put back at once at the same temperature as before. After the insects had been kept for a few days, however, a warm plate (Tattersfield & Potter, 1943) was used, with the beetle species, to make counting easier.

The percentages affected were calculated by the method of Tattersfield, Gimingham & Morris (1925), and were corrected for deaths in the controls by the method of Abbott (1925). This method for calculating the percentage affected did not distinguish between paralysed and dead insects. Both were taken as 'affected', although their

relative proportions changed continuously throughout each test. Thus, temperature coefficients of toxic action, measured soon after treatment, were most probably temperature coefficients of paralysis only; and those at the end of the test, coefficients of kill, with continuous gradation between.

Probit lines were fitted to the points obtained, and values for ED 50 (Finney, 1952), were read from the lines. ED 50's were used throughout to measure the effects of temperature, even though the probit lines obtained at the two temperatures were not always parallel. The lines were usually fitted by eye, but when there was doubt about the significance of the effect of temperature, the lines were often fitted to the combined sets of points from replicate tests by the method of Finney (1952); and a *t*-test was used to assess the significance of the difference between ED 50's. Thus, the accuracy of the figures given below for ED 50's and temperature coefficients varies, depending on whether the lines were fitted by eye or by calculation, but this does not affect the general conclusions.

Each probit line was based on about 5 points, i.e. on about 300 *O. surinamensis* or *T. castaneum*, 150 *T. molitor* or 200 *M. domestica*. Each ED 50 is based on some multiple of one of these figures, according to the number of replicate tests it represents.

The changes in ED 50, and in temperature coefficient, were followed for as long as possible, but the period for which this could be done was often restricted. The poison sometimes acted so slowly at the lower post-treatment temperature that no ED 50 could be obtained until several days had passed. Further, the duration of each test, especially those with house flies, was often limited by the gradually increasing percentage of insects dying in the controls. The natural life of the insects could be prolonged by feeding them (*T. molitor* and *M. domestica*), or by keeping them at 100% R.H. (*O. surinamensis* and *T. castaneum*), but tests were not usually followed beyond the time when the proportion dead in the controls exceeded 40%, even though the end-point had not been reached. However, the advantage of using 100% R.H. was slight, and when both uncontrolled and 100% R.H. were used in otherwise identical tests, the results of the two series were always very similar; they were therefore combined to give the figures or lines shown below for 'both humidities'.

Figs. 1–10 show the results of most groups of tests; logarithms of geometric means of ED 50's from replicate tests are plotted against time, or occasionally logarithms of time, after treatment. However, in Fig. 4 the logarithms of ED 50's from replicate tests are plotted separately against the logarithms of time after treatment. The units of ED 50 are w/v% applied (*O. surinamensis*) or μg./insect (all other species).

End-points (the times at which the ED 50's reached steady values) were estimated by eye from the curves; the estimates may therefore have subjective errors, although these could hardly affect the general argument.

Pairs of ED 50's calculated by the method mentioned above are enclosed in rectangles or are joined by vertical arrows. The rectangles show the pairs that do not differ significantly from each other (*t*-test); the vertical arrows show those that do. Other pairs of points are unmarked. It is assumed that in each series, temperature coefficients (ratios of geometric means of ED 50's) larger than those marked by vertical arrows also correspond to significantly different ED 50's.

The figures beside the points on the lines are the percentages dead in the corresponding control batches, if they exceeded 10%.

Table 1 summarizes the results of all the tests in which the observed temperature coefficients were very small; these results are not shown in the Figures. The ED 50's, on the right of the table, are in the same units as in the Figures. They are the geometric means from replicate tests (eye-fitted lines) or the calculated values (see p. 272), on the first and last occasion when a numerical value could be calculated for the temperature coefficient. The coefficients, preceded by + or − (see p. 269), are shown in italics between the corresponding pairs of ED 50's. Those which are known, from the *t*-tests, to correspond to significant differences in toxicity are marked by asterisks; others are unmarked.

RESULTS

The results are outlined in the following sections, poison by poison, and the *general conclusions are given in the last paragraph* of each section.

Rotenone

The fumigant action of rotenone was not tested.

Fig. 1 shows the combined results of six tests on *O. surinamensis* at both humidities, and of one on *T. molitor*, which could not be repeated because of an unexpected increase in the resistance of our strain of *T. molitor* to rotenone.

The curves from the two species show that the same general sequence was followed at each temperature. When the insects were first counted, some were measurably affected by the poison. After this, and possibly for some time before, they recovered to some extent, as is shown in Fig. 1 by the initial increases in ED 50. Later still, the insects (except the *O. surinamensis* at 10° C.) began to become irreversibly paralysed, or to die, so that the ED 50's decreased again. The ED 50 for the *O. surinamensis* at 10° C. might have decreased again had it been possible to keep them longer. None of the end-points was reached for certain, but the sequence of knockdown–recovery–death occurred more quickly at 28° C. than at 10° C., and from this it seems likely that the end-point would be reached sooner at 28 than 10° C. The temperature coefficients changed from −2·0 (paralysis) after 1 day to +1·3 after 6 days (*O. surinamensis*), and from −8·8 (paralysis) after 2 days to +19 after 16 days (*T. molitor*). No figures can be given for the end-point temperature coefficients.

Thus, an increase in post-treatment temperature probably increased the speed of action of rotenone on *O. surinamensis* and *T. molitor*. As the end-points were not reached, there is no certain evidence about the effect on the ultimate toxicity; as increase in temperature increased the speed of the sequence knockdown–recovery–death, the shape of the curve was affected.

'*Dimetan*'

'Dimetan' had no fumigant action, at either 10 or 28° C., on *O. surinamensis* or *M. domestica* after 2 days.

Table 1 summarizes the combined results of three tests on *O. surinamensis* at 100% R.H. only. Fig. 2 shows the combined results of two tests on *M. domestica*.

Temperature had little apparent effect on the toxicity to *O. surinamensis*. The end-points were rather ill-defined, but were reached after 1 or 2 days at either temperature. The temperature coefficient was consistently small and positive.

The results with *M. domestica* resembled those with rotenone on *O. surinamensis* and *T. molitor*. The same sequence of knockdown–recovery–death, which was slower at 10° C. than at 28° C. in the tests of rotenone, was evident here at 10° C. but not at 28° C. (Fig. 2); however, the changes were much smaller than with rotenone. The end-points were reached after about 3 days at 28° C. but not even after 12 days at 10° C.

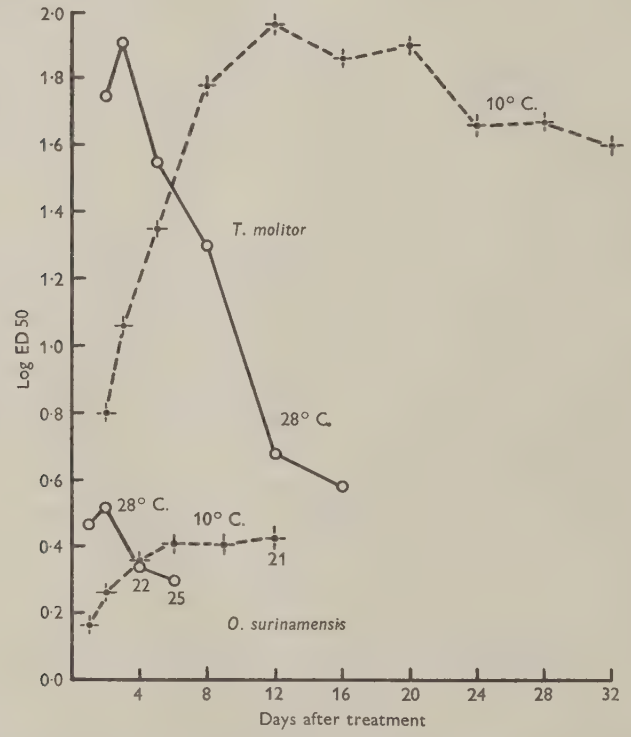

Fig. 1. Combined results of one test of rotenone on *T. molitor* and six on *O. surinamensis*. Vertical scale: log ED 50 as μg./insect (*T. molitor*) or as w/v % × 10^4 (*O. surinamensis*.) See also notes on pp. 272–273.

The temperature coefficient changed from −1·7 after 1 day to 1·0 after 3 days and then to −1·2 after 5 days, but it did not become positive; unlike those of rotenone, the two lines do not cross. No figure can be given for the end-point temperature coefficient, but extrapolation of the 28° C. line suggests a slightly larger negative value than 1·2.

Thus, the effects of temperature on the toxicity of 'Dimetan' were quite small,

especially on *O. surinamensis*. However, in the tests on *M. domestica*, an increase in post-treatment temperature increased the speed of action and probably slightly decreased the ultimate toxicity; the shape of the curve was also affected.

2-*Bromomercurithiophen*

This poison had no fumigant action, at either 10 or 28° C., on *O. surinamensis* after 2 days or on *T. molitor* after 12 days.

Fig. 3 shows the combined results of three tests of crystals (mean size $52 \times 32 \mu$) on *O. surinamensis* at 100% R.H. only. (In these tests, the higher temperature was 23° C., not 28° C.) Table 1 summarizes the combined results of four tests of colloid on *O. surinamensis* at both humidities, and two tests on *T. molitor*.

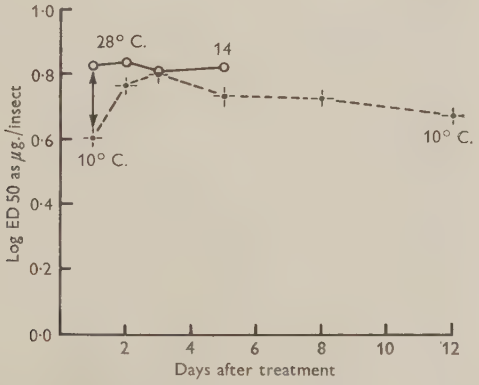

Fig. 2. Combined results of two tests of 'Dimetan' on *M. domestica*. See also notes on pp. 272–273.

Temperature had little apparent effect on the toxicity of colloidal 2-bromomercurithiophen to *O. surinamensis*; had it been possible to count the insects sooner than 1 day after treatment, larger differences might have been found.

In contrast, temperature affected the toxicity of crystals to *O. surinamensis* much more (Fig. 3; cf. McIntosh, 1957*a*, *b*); the temperature coefficient changed from +11 after 1 day to an end-point value of −1·2 after 7 days. The end-points were reached after about 7 days at each temperature. If it is assumed that, before the insects were first counted, the ED 50's were very large indeed, so that the early parts of the lines would be almost vertical, then the shape of the curve was clearly affected by temperature. The curvature of the line was greater at 23° C. than at 10° C.; the change from almost vertical to horizontal occurred over a much shorter time at 23° C. than at 10° C.

Although it may not be clear at once from Table 1, the results with *T. molitor* resembled those with crystals on *O. surinamensis*. The ED 50 after 1 day at 28° C. was 64·8 μg./insect, but the speed of action at 10° C. was so low that no figure for ED 50 could be obtained until the 4th day, when the temperature coefficient was

Table 1. *Results of tests in which the observed temperature coefficients were consistently small. ED 50's are given as w/v %* (Oryzaephilus surinamensis) *or as μg./insect* (Tenebrio molitor). *See also notes on p. 273.*

Poison	Test species	No. of replicate tests	Post-treatment temperatures (°C.)	Mean time to reach end-point (days)	Maximum duration of test (days)	Final control kill (%)	Mean ED 50's and *temperature coefficients* (after n days) First	Last
'Dimetan'	*O. surinamensis*	3	28	1	6	43	0·22 *+1·4* (1)	0·18 *+1·5* (6)
			10	1	6	3	0·30	0·27
2-Bromomercuri-thiophen	*O. surinamensis* (colloid only)	4	28	2	6	36	0·012 *+1·4* (1)	0·011 *−1·7* (6)
			10	>12	12	24	0·017	0·0063
	T. molitor	2	28	16	24	9	33·2 *+1·5** (4)	16·2 *+2·1** (24)
			10	>24	24	2	49·2	33·4
'Valone'	*T. molitor*	2	28	~ 5	12	4	17·7 *+1·3* (1)	12·3 *1·0* (12)
			10	~12	12	0	22·6	12·3

* Differences between ED 50's significant by *t*-test.

+1·5. No exact figure can be given for the temperature coefficient before the 4th day, but it was clearly very large and there is no doubt that the ED 50–time curves would take the same general form as those in Fig. 3. The apparent increase in temperature coefficient between the 4th and 24th days is very small compared with the decrease before the 4th day.

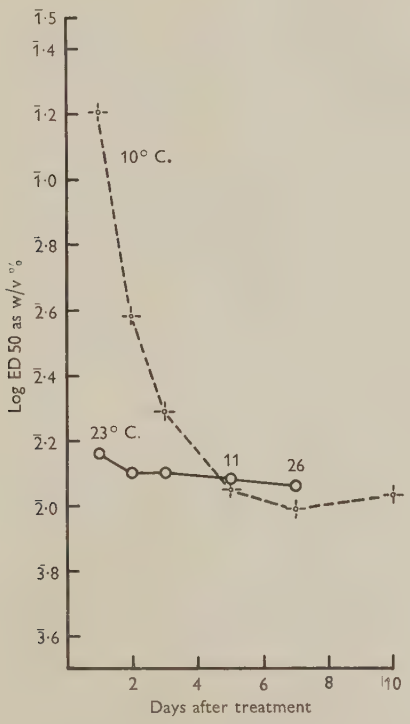

Fig. 3. Combined results of three tests of crystals of 2-bromomercurithiophen on *O. surinamensis*. See also notes on pp. 272–273.

The results obtained with colloidal 2-bromomercurithiophen differed in degree from those with crystals on *O. surinamensis*, and from those on *T. molitor*, but they all followed the same general pattern. All ED 50's decreased steadily as time passed, and the observed decreases were greater at 10° C. than at 23° C. or 28° C. In the tests with crystals on *O. surinamensis*, the end-points were reached at about the same time at each temperature; in the other tests, the end-points were reached at 28° C. but not at 10° C. The temperature coefficients were all initially positive. They decreased in size as time passed, and occasionally became negative; the end-point or last values were small and positive or negative, according to the species.

Thus, an increase in post-treatment temperature probably increased the speed of action of 2-bromomercurithiophen on *O. surinamensis* and *T. molitor*, and affected the shape of the curve. The ultimate toxicities were apparently unaffected.

'Valone'

'Valone' had no fumigant action, at either 10 or 28° C., on *T. castaneum* after 10 days, on *T. molitor* after 12 days, or on *M. domestica* after 6 days at 10° C. or 1 day at 28° C.

Table 1 summarizes the combined results of two tests on *T. molitor*. Figs. 4 and 5 show the combined results of three tests on *T. castaneum* at uncontrolled R.H. only, and two on *M. domestica*, respectively. In the three tests with *T. castaneum*, the intervals at which the insects were counted were not the same in each test and, as the variation in ED 50 from test to test was greater than usual, successive means of ED 50's were seldom really comparable with each other. The lines for the three tests are therefore drawn separately in Fig. 4; and, for greater clarity, the logarithms of the times after treatment are used instead of the times themselves.

'Valone' affected *T. castaneum* so quickly that the end-point at 28° C. was reached after about 12 hr. (test B, Fig. 4); in tests A and C, no change was found in the ED 50's at 28° C., presumably because the insects were not counted soon enough. At 10° C., the end-point was reached after about 3 days. The temperature coefficient was +5·4 after 8 hr. (test B), but the end-point temperature coefficient varied in size and significance from test to test; the largest value found was −4·8 or more after 2 days (test B), and the smallest −1·4 after 7 days (test A). There was no clear evidence about the effect of temperature on the shape of the curve.

The results with *M. domestica* were similar (Fig. 5). The end-points were reached after about 12 hr. (28° C.) and 3 days (10° C.); the temperature coefficient changed from +2·5 after 6 hr. to an end-point value of −1·6 after 5 days. The curvature of the lines (see p. 275) was, if anything, greater at 28° C. than at 10° C.

Temperature had little apparent effect on the toxicity of 'Valone' to *T. molitor* (Table 1), although larger changes in ED 50 and temperature coefficient would no doubt have been found had the insects been counted sooner than 1 day after treatment.

The results with all three species followed the same general pattern. All ED 50's decreased steadily as time passed, and the observed decreases were greater at 10° C. than at 28° C. The end-points were reached sooner at 28° C. than at 10° C., although they were rather ill defined in the tests on *T. molitor*. The temperature coefficients were all initially positive. They decreased in size as time passed; in the tests on *T. molitor*, the last value obtained was 1·0; in the tests on *M. domestica*, and in some of the tests on *T. castaneum*, the temperature coefficients became significantly negative.

Thus, an increase in post-treatment temperature increased the speed of action of 'Valone' on *T. castaneum*, *M. domestica* and *T. molitor* and, in some tests, decreased the ultimate toxicity; in others, the ultimate toxicity was apparently unaffected. There was very slight evidence that the shape of the curve was affected.

α-Chlordane

α-Chlordane had no fumigant action, at either 10 or 28° C., on *T. castaneum* after 6 days, on *T. molitor* after 12 days, or on *M. domestica* after 6 days at 10° C. or 1 day at 28° C.

Fig. 6 shows the combined results of two tests on *T. castaneum* at 100% R.H. only, two on *T. molitor* and two on *M. domestica*.

The speed of action of α-chlordane on *T. castaneum* and *T. molitor* was so low at 10° C. that no ED 50 could be obtained until the 5th day (*T. castaneum*) or the 2nd day

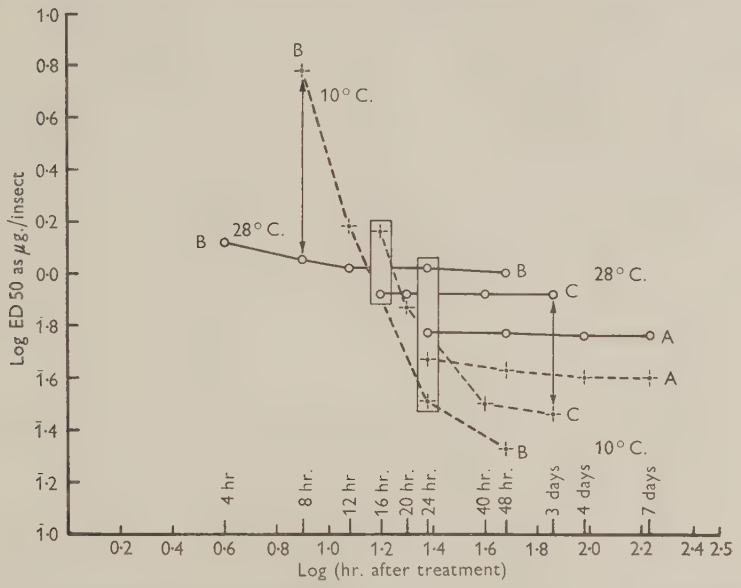

Fig. 4. Separate results of three tests (A, B and C) of 'Valone' on *T. castaneum*.
See also notes on pp. 272–273 and 278.

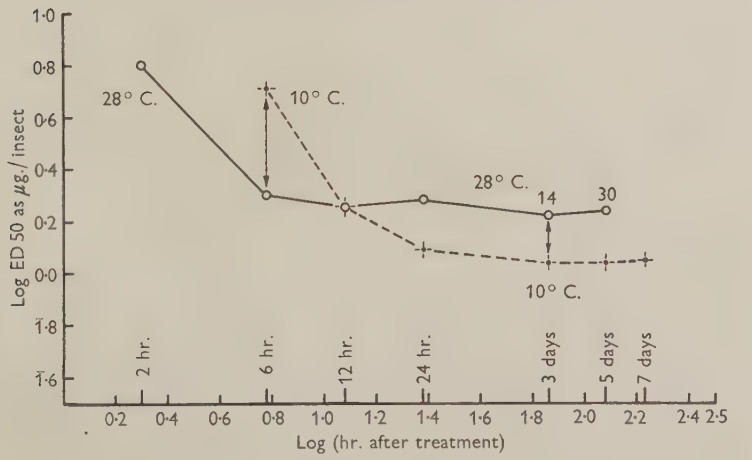

Fig. 5. Combined results of two tests of 'Valone' on *M. domestica*.
See also notes on pp. 272–273.

(*T. molitor*), and the end-points were not reached, with either species, even after 24 days. At 28° C. the end-points were reached after about 20 days (*T. castaneum*) and 12 days (*T. molitor*). The temperature coefficients before the 5th or 2nd days were positive and very large. They changed from +3·3 after 5 days to −4·0 after 24 days (*T. castaneum*), and from +7·8 after 2 days to −3·5 after 24 days (*T. molitor*). No figures can be given for the end-point temperature coefficients, but they were probably larger than these values.

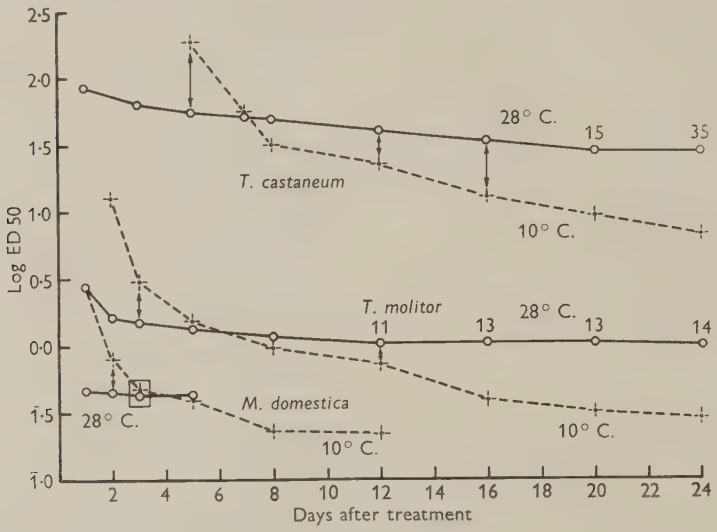

Fig. 6. Combined results of two tests of α-chlordane on *T. castaneum*, two on *T. molitor* and two on *M. domestica*. Vertical scale: log ED50 as μg./insect × 100 (*T. castaneum*), or as μg./insect (*T. molitor* and *M. domestica*). See also notes on pp. 272–273.

Results with *M. domestica* were similar in form, but the speed of action was greater. End-points were reached after 3 days (28° C.) and 8 days (10° C.). The temperature coefficient changed from +6·1 after 1 day to −1·1 after 5 days. Again, no figure can be given for the end-point temperature coefficient, but extrapolation of the 28° C. line suggests a larger negative value than 1·1.

The results with all three species followed the same general pattern. All ED50's decreased steadily as time passed, and the observed decreases were greater at 10° C. than at 28° C. With *M. domestica*, the end-point was reached sooner at 28° C. than at 10° C.; with the other species, end-points were reached at 28° C. but not at 10° C. The temperature coefficients were all initially large and positive; they decreased in size as time passed, becoming negative and, in the tests on *T. castaneum* and *T. molitor*, quite large. The curvature of the lines (see p. 275) was greater at 28° C. than at 10° C.

Thus, an increase in post-treatment temperature increased the speed of action of α-chlordane on *T. castaneum*, *T. molitor* and *M. domestica*, decreased its ultimate toxicity and affected the shape of the curve.

Toxaphene

Toxaphene had no fumigant action, at either 10 or 28° C., on *T. castaneum*, or at 10° C. on *T. molitor*, after 12 days. Only 9% of *T. molitor* were affected after 12 days at 28° C.

Fig. 7 shows the combined results of two tests on *T. castaneum* at 100% R.H. only, and two on *T. molitor*.

The speed of action on *T. castaneum* was so low at 10° C. that no ED 50 could be obtained until the 5th day; and the end-points were not reached even after 24 days (*T. castaneum*) or 20 days (*T. molitor*). At 28° C. the end-points were reached after 16 days (*T. castaneum*) and 9 days (*T. molitor*). The temperature coefficient for *T. castaneum* was positive and very large before the 5th day; the coefficients changed

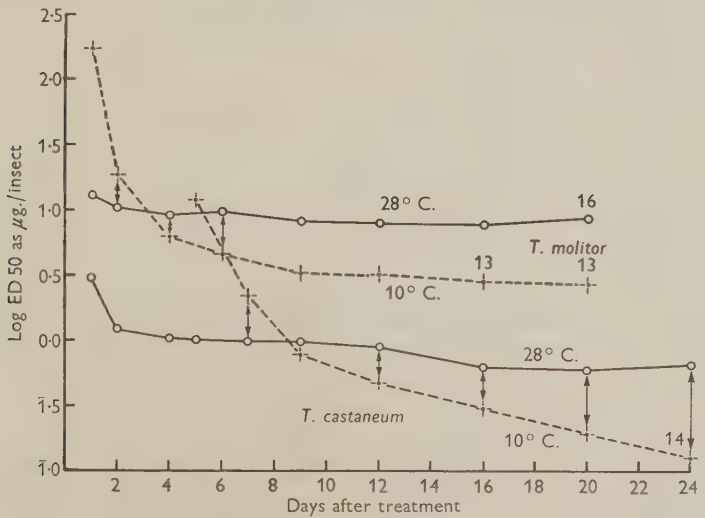

Fig. 7. Combined results of two tests of toxaphene on *T. molitor* and two on *T. castaneum*. See also notes on pp. 272–273.

from +12 after 5 days to −5·1 after 24 days (*T. castaneum*), and from +13 after 1 day to −3·2 after 20 days (*T. molitor*). No figures can be given for the end-point temperature coefficients, but they were probably larger than these values. The curvature of the lines (see p. 275) was greater at 28° C. than at 10° C.

The results given by toxaphene with the two species are similar to each other and to those with α-chlordane on the same species. Thus, an increase in post-treatment temperature increased the speed of action of toxaphene on *T. castaneum* and *T. molitor*, decreased its ultimate toxicity, and affected the shape of the curve.

DDT

The fumigant action of DDT was not tested.

Figs. 8–10 show the combined results of two tests on *O. surinamensis* at 100% R.H. only, three on *M. domestica*, two on *T. molitor*, and six on *T. castaneum* at both humidities.

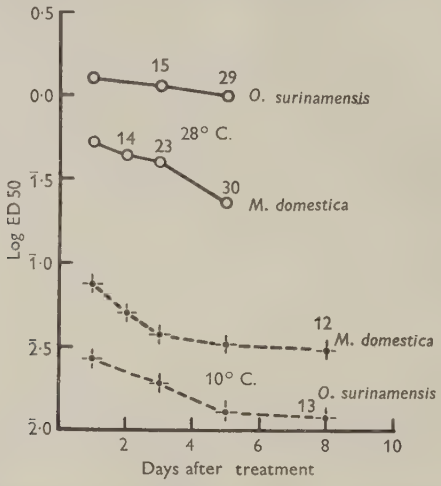

Fig. 8. Combined results of two tests of DDT on *O. surinamensis* and three on *M. domestica*. Vertical scale: log ED 50 as w/v % × 100 (*O. surinamensis*) or as μg./insect (*M. domestica*). See also notes on pp. 272–273.

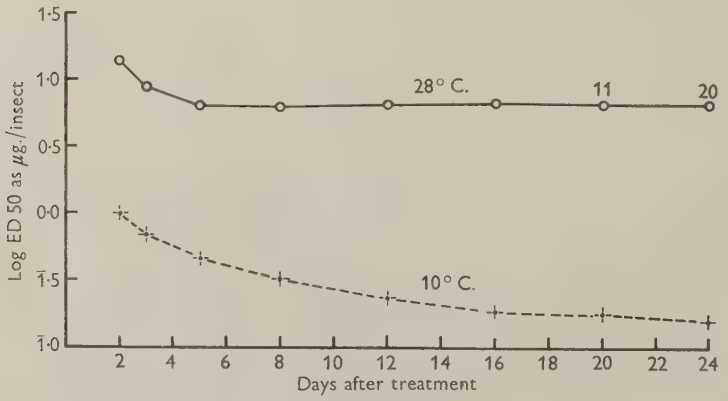

Fig. 9. Combined results of two tests of DDT on *T. molitor*. See also notes on pp. 272–273.

End-points were not reached for certain at either temperature in the tests on *O. surinamensis* and *M. domestica*. In the tests on *T. castaneum*, the end-point was reached after 16 days at 10° C. but not even after 24 days at 28° C.; in the tests on *T. molitor*,

the end-points were reached after 5 days at 28° C., but not even after 24 days at 10° C.

In the tests on *O. surinamensis* and *T. molitor*, the temperature coefficients increased from −47 after 1 day to −75 after 5 days (*O. surinamensis*), and from −14 after 2 days to −41 after 24 days (*T. molitor*). In the tests on *T. castaneum*, it increased from −6·5 after 1 day to −13 after 16 days and then decreased to −3·9 after 24 days. In the tests on *M. domestica*, the temperature coefficients remained almost unchanged in size; the mean value was −8·3. Apart from this value, no figures can be given for the end-point temperature coefficients.

(Two tests on *T. molitor* by injection, which are not described above, gave results similar to those from the contact tests on *M. domestica*; end-points were not reached for certain at either temperature, and the temperature coefficient (−12·5) was almost constant.)

Thus, all ED 50's decreased steadily as time passed. However, DDT differed from the other poisons tested in that no general statement can be made about the

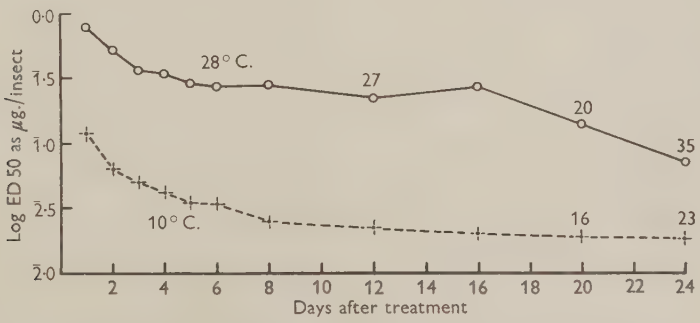

Fig. 10. Combined results of six tests of DDT on *T. castaneum*.
See also notes on pp. 272–273.

effect of temperature on its speed of action, or about the changes in size of its temperature coefficients with the passage of time, or about the effects of temperature on the shape of the curve. The temperature coefficients remained negative for the duration of the tests, and were larger than any negative coefficient obtained with the other poisons.

Thus, the main effect of an increase in post-treatment temperature was to decrease the toxicity of topically applied or injected DDT at all stages of the tests.

DISCUSSION

Although many sets of results given here are incomplete, some general conclusions can be drawn. It is assumed that all ED 50's decreased from very high values during the very early stages of the tests, and finally reached steady values at the end-points. For practical reasons it was impossible to follow these changes over their entire course, especially during the early stages. However, the speed and manner at which such

changes occurred clearly depended on the post-treatment temperature, and on the poison.

(1) The ED 50's of the poisons with knockdown properties (rotenone and 'Dimetan') did not of course decrease steadily as time passed. Their speeds of action were probably greater at 28° C. than at 10° C., but this is by no means certain from the results of the tests with rotenone (Fig. 1), because the end-points were not reached at either temperature. There was no certain evidence about the effect of temperature on the ultimate toxicity.

It is clearer from the results with rotenone and 'Dimetan' than from those with the other poisons that the early (negative) coefficients were coefficients of paralysis, and that the later (positive) coefficients were based on kill rather than paralysis.

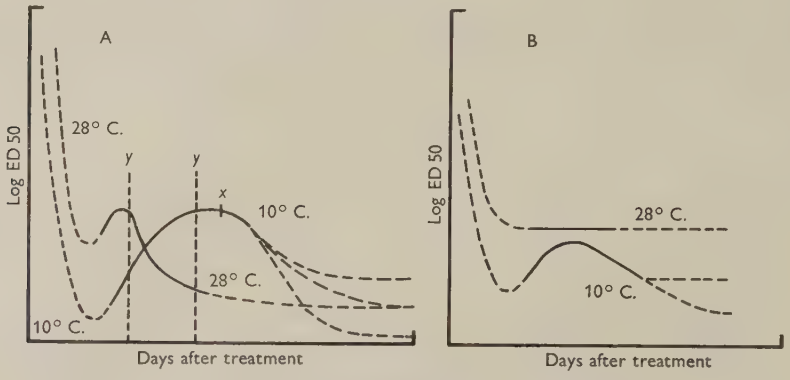

Fig. 11. Completed curves, showing possible courses of poisoning by rotenone (A) and 'Dimetan' (B). For explanation see p. 284, §(1).

The results given by these two poisons were more fragmentary than the others, but we can suppose that the complete curves would take one of the forms shown in Fig. 11 A (rotenone) and 11 B ('Dimetan'). The unbroken curves correspond to those obtained experimentally (Figs. 1, 2). The mark at *x* in Fig. 11 A shows the stage at which the tests of rotenone on *O. surinamensis* at 10° C. were ended; the broken vertical lines *yy* show the time limits of a somewhat similar fragmentary test by injection of colloidal rotenone into *T. molitor* (Fig. 3 of McIntosh, 1957*a*; vertical scales reversed).

(2) The results of the tests of 2-bromomercurithiophen, 'Valone', α-chlordane and toxaphene, which have no knockdown properties, were very similar to each other, and all followed a general pattern. In each series, the ED 50's decreased steadily as time passed, reaching or approaching final steady values at the end-points; the observed decreases in ED 50 were greater at the lower temperature; the speeds of action, which were greater with 'Valone' than with any of the other poisons, were nearly always greater at the higher temperature; the initial temperature coefficients (probably of paralysis) were positive, but changed to smaller or negative coefficients (probably of kill) as time passed; and the ultimate toxicities were either unaffected or decreased by the increase in post-treatment temperature.

In the tests of colloidal 2-bromomercurithiophen on *O. surinamensis* and of 'Valone' on *T. molitor* (Table 1), the effects of time and temperature on toxicity were slight, but the results did not differ in essentials from those of the same poisons on other species.

(3) The results of the tests with DDT in this paper conform with the above general pattern in that, in each series, the ED 50's decreased steadily as time passed, and the ultimate toxicity was decreased by increase in post-treatment temperature. However, the temperature coefficients were large and consistently negative throughout all the tests. Earlier counting might, of course, have revealed initially positive coefficients, so that the results with DDT would then resemble those with α-chlordane or toxaphene (Figs. 6, 7); but the great difficulty in classifying DDT-poisoned insects during the early stages of a test makes confirmation of this impossible. However, such initial positive coefficients, which were almost certainly coefficients of paralysis, have sometimes been found. Menn, Benjamini and Hoskins (1957), Barker (1957) and Das (1961), using DDT-resistant *M. domestica* and normal *T. molitor* in the post-treatment temperature ranges 15–25° C. and 5–15° C. (*M. domestica*) or 2–6° C. (*T. molitor*), found positive coefficients after 1 day (*M. domestica*) or 12 days (*T. molitor*), but negative coefficients after 2 days (*M. domestica*) or 20 days (*T. molitor*). (In the tests on *T. molitor*, this change occurred only at low doses; at higher doses the behaviour was more similar to that of crystals of 2-bromomercurithiophen on *O. surinamensis* (Fig. 3).)

Thus, although our results with DDT in the range 10–28° C. were distinct from those with 2-bromomercurithiophen, 'Valone', α-chlordane and toxaphene in the same temperature range, DDT can under extreme conditions conform to the general pattern (§ (2), above), showing the trend common to all these poisons: an initially positive temperature coefficient, changing towards negative as time passes (see also pp. 287–288).

(4) The shapes of the curves relating ED 50 to time are not easy to define, especially as many of the curves are incomplete. As the general effect of an increase in post-treatment temperature was to increase the speed of action, it also generally increased the curvature of the ED 50–time lines. (In some of the Figures (e.g. Fig. 3), this is best seen by extrapolating the lines to zero time; it is assumed that each ED 50 decreased from a very high value during the earliest stages of the tests.)

Thus, an increase in post-treatment temperature increased the curvature of the second bends in the lines obtained with rotenone (Fig. 11 A). Similarly, it either did not affect (Table 1, Fig. 4) or, more commonly, increased the curvature (Figs. 3, 5–7) of the lines obtained with 2-bromomercurithiophen, 'Valone', α-chlordane and toxaphene; the end-point ED 50's were reached later, and were approached more gradually, at the lower temperature. With 'Dimetan' the shape of the curve was clearly affected (Figs. 2, 11 B); with DDT it was evidently unaffected (Figs. 8–10).

(5) Munson (1953*a*, *b*) and Munson, Padilla & Weissmann (1954) showed that an increase in pre-treatment temperature from 23 to 34° C. for 2 weeks decreased the resistance of American cockroach nymphs to DDT and chlordane, the post-treatment temperature being constant (23° C.). The 2-week increase in pre-treatment temperature also decreased the iodine number of the total lipids of the nymphs. According to

Munson, the decreased resistance of the 34° C. nymphs was caused by an increase in the distribution coefficient of DDT between lipid at the site of action and general lipids.

Such changes in the nature of the lipids may have occurred in our tests, many of which lasted for about 3 weeks. However, these changes could not explain the general trend for an initial positive post-treatment temperature coefficient to change to negative; if Munson is correct, they would in fact cause a change in the opposite direction. Further, the change in temperature coefficient from positive to negative could occur within 1 day (Figs. 4, 5), which is probably too soon for any change in the iodine numbers to have occurred.

(6) The general nature of the effects of post-treatment temperature and time on the toxicity of a poison to any species was characteristic of the poison and not of the species. Thus, although there were of course differences in degree, insects of two species could recover, at either temperature, from the initial paralysis produced by rotenone (Fig. 1); the speed of action of 'Valone' on three species was greater, at either temperature, than those of α-chlordane or DDT on the same species (Table 1, Figs. 4–6, 8–10); the temperature coefficients of α-chlordane on three species changed from positive to negative (Fig. 6); and the temperature coefficients of DDT on four species were consistently large and negative (Figs. 8–10).

The variation in degree of response to temperature from species to species was not the same for different poisons. Thus the changes found in the tests of rotenone were much greater on *T. molitor* than on *O. surinamensis* (Fig. 1), but in the tests of DDT the reverse was true (Figs. 8, 9). Similarly, the observed post-treatment temperature coefficients of DDT were greater on *T. molitor* than on *T. castaneum* (Figs. 9–10); the distinction between these species was not evident in the tests of α-chlordane (Fig. 6).

(7) No single figure, and often no single sign, is enough to define the temperature coefficient of toxic action of a poison under any one set of conditions. Temperature coefficients, which can change continuously in quality from coefficients of paralysis to coefficients of kill, may also change in sign from positive to negative, or vice versa, as time passes.

The end-point temperature coefficient is the temperature coefficient of kill, which cannot be calculated until both end-points have been reached. In tests which give results like those of rotenone (Fig. 11A), the coefficient of maximum paralysis (negative) is quite distinct from the coefficient of kill, and may be defined by a numerical value and sign. With other types of poison, the temperature coefficient of paralysis is not sharply defined, and no numerical value can be given for it. However, it is probably safe to assume that the sign of the temperature coefficient of paralysis was the same as that of the earliest measurable value, i.e. positive for 2-bromomercurithiophen, 'Valone', α-chlordane and toxaphene (Table 1, Figs. 3–7) but negative, in our tests, for DDT (Figs. 8–10). The results with 'Dimetan' (Table 1, Fig. 2, 11B) are too meagre for any general statement to be made.

(8) Similarly, no single figure is enough to define the relative toxicity (ratio of ED50's) of two poisons tested in the same way on the same species (Beard, 1949).

(9) The proportion of insects dying in the control batches was nearly always increased by increase in post-treatment temperature; it had often reached about 30% by the end of the tests at 28° C. These high control death rates no doubt decreased the

reliability of the corresponding figures for ED 50, but were unavoidable in tests which were followed to the end-points. However, the general conclusion would scarcely have been different had the control death rates been lower.

Negative post-treatment temperature coefficient of DDT

Although initial transient positive coefficients (of paralysis) may occasionally be found with DDT (§ (3), above), more permanent positive coefficients are sometimes found. This happens under rather special conditions, which mask the basic negative coefficient. Positive continuous treatment or post-treatment temperature coefficients (Pradhan & Mundkur, 1957) may thus arise from the pick-up effect (Lindquist, Madden & Schroeder, 1946; Pradhan, 1949); from the 'dose effect' (Fan, Cheng & Glenn Richards, 1948; Das & Needham, 1961); or when the temperature range is over 30° C. (Barker, 1957; Pradhan & Rangarao, 1957). The high-temperature effect may in fact be a special case of the dose effect, the very high temperature, like a very high dose, greatly increasing the rate of entry of DDT into the insects.

However, the basic negative post-treatment coefficient remains. Although the difference between DDT and the other non-knockdown poisons may theoretically be one of degree only (§ (3), above), our results emphasize that in the range 10–28° C. the difference is so large as to distinguish DDT quite clearly from the other poisons. Its temperature coefficient was always consistently large and negative, and its property of 'reversibility of action' (Das & Needham, 1961) is not known with other insecticides.

Thus, the effects of temperature on the toxic action of DDT seem to be unique. Our results in no way explain them; but it may be useful to point out that most of the existing explanations of the negative post-treatment temperature coefficient of DDT are certainly wrong. Hurst (1949) suggested that the rate of DDT activity has a small (positive) temperature coefficient, that a (general) rate of metabolism, which can overcome the effects of DDT, has a larger (positive) coefficient, and that the observed effect is the result of the balance between these two. In a similar explanation, Pradhan (1950) substituted 'entry' for activity and 'resistance' for rate of metabolism; this may have some value in explaining the dose effect (Das & Needham, 1961). Munson *et al.* (1954) suggested that changes in post-treatment temperature can affect the distribution of DDT amongst body lipids, and therefore its toxicity; this parallels their explanation of the effects of pre-treatment temperature (§ (5), above). In two reviews, Kearns (1956) and Hoffman (1956) repeated a misquotation by Munson *et al.* (1954) of Hurst's paper (1949; see also Leeper, 1948). According to Munson *et al.*, Hurst suggested that the reversibility of action is caused by DDT being 'thrown out' of solution in the cuticular fats at the lower temperature; it is then available for toxic action. This idea was even extended by Kearns to explain the negative post-treatment temperature coefficient itself. If the cause did lie in 'throwing out', DDT could never affect insects which are kept continuously at a low temperature after treatment, because it could never supersaturate the fats. Finally, Busvine (1957) proposed an explanation based on Ferguson's principle (1939).

None of these explanations is supported by any direct evidence. Most of them are based on the general properties (usually physical) of fat-soluble poisons, and all fail because they do not explain why DDT should behave differently from other poisons.

According to the explanations, all poisons should behave similarly. There is little or no support for this view, even from experiments that were designed to test it (Pradhan & Rangarao, 1957). In a realistic review, Yamasaki & Ishii (1954)* discussed the failings of two other explanations—those depending on the cuticle (Fan *et al.* 1948), and on detoxification (see also Wiesmann, 1955; Barker, 1957; Menn, Benjamini & Hoskins, 1957). These reviewers also showed that the direct effect of DDT on nerve cells has a negative temperature coefficient, and shows reversibility of action; and they suggested that the behaviour of DDT towards intact insects is caused by such action. It is hard to believe that this is not so, and that it is this action on the nerve cells which differentiates DDT from other poisons. The effects of temperature may similarly be used to test or confirm theories about the mechanism of action of other poisons.

We would like to thank Dr C. Potter and other colleagues for criticisms of this paper.

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The epidemiology of apple scab (*Venturia inaequalis* (Cke.) Wint.)

I. Frequency of airborne spores in orchards

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SUMMARY

The number of ascospores caught by automatic volumetric spore traps differed widely in six orchards where the air was sampled in some or all of the years 1955 to 1959. To compare amounts of inoculum in different orchards the total catch in each was expressed as the 'relative ascospore dose'. The reason for the much smaller doses in the later years is uncertain. The results confirmed earlier work on the phenology of ascospore liberation and stressed the practical need to decrease their number as much as possible.

Because conidia have previously been trapped almost only on wet and windy days, they have been considered to be rarely air-dispersed. Our apparatus, which efficiently traps dry conidia but catches water-borne ones only when in very small droplets, caught airborne conidia in all orchards when they operated during summer. Traps among heavily infected, unsprayed trees caught most conidia around noon on warm dry days and the highest concentration measured exceeded 1300 per m.^{3} of air.

INTRODUCTION

This is the first of several papers describing the extension of aerobiological studies on *Venturia inaequalis* (Cooke) Wint., started in Bramley's Seedling orchards at Wisbech, Cambs. in 1953 (Hirst, Storey, Ward & Wilcox, 1955), into a general study of the epidemiology of apple scab. Concentrations of airborne conidia and ascospores were measured with power-operated suction traps (Hirst, 1952), which allow the concentration of even small spores to be estimated accurately each hour. These traps are ill-adapted to measuring the viability of spores or their dispersal in splashed or dripping water, but much can nevertheless be learnt from air-dispersal alone.

METHODS OF TRAPPING SPORES

An air stream of 10 l./min. is drawn through the trap (Hirst, 1952) and the spores are impacted on a sticky-surfaced microscope slide moved slowly behind an orifice (14 × 2 mm. cross-section) directed into the wind 2 m. above ground. The slide is moved at 2 mm. per hour past an orifice 2 mm. wide, so the deposit takes an hour to accumulate. The slides were prepared and mounted as previously (see Hirst, 1953,

p. 377), except that 10% of paraffin wax m.p. 54° C. was added to the 'Vaseline' adhesive.

Counts on 'short traverses' (14·0 × 0·27 mm.) across the deposit were used to estimate spore concentration and no correction was applied for the efficiency of the trap (see Hirst, 1953, p. 378–9). Had an area equal to the whole cross-section of the orifice (28 mm.^{2}) been counted, the 'detection threshold' (Hirst, 1959), or least concentration the trap could reliably detect, would have been 1·7 spores per m.^{3} of air, but the scanning procedure adopted gave a detection threshold of 12 spores per m.^{3} of air. Smaller concentrations than this would have been detected only by chance, but these could still represent many spores in a large orchard; for example, 12 per m.^{3} of air total over a million in the lowest 12 ft. of a 5-acre orchard.

Table 1. *Trapping efficiency and estimated detection threshold concentration (spores per m.^{3} of air) of suction trap and sticky microscope slide inclined at 45°, assuming exposure for 1 hr. and complete count of 28 mm.^{2} of spore deposit*

	Wind speed ... (m./sec.)	0·5	1·1	1·75	3·2	5·5	9·5
	Microscope slide inclined at 45° (Gregory & Stedman, 1953 and unpublished)						
Lycopodium	Efficiency (%)	3·0	5·0	5·2	10·0	12·7	19·7
	Detection threshold	940	260	150	40	20	8
Erysiphe	Efficiency (%)	1·4	0·9	0·9	1·5	3·7	7·7
	Detection threshold	2000	1500	860	340	70	10
Ustilago	Efficiency (%)	0·1	0·1	0·04	0·3	0·4	0·3
	Detection threshold	28,000	13,000	20,000	1400	640	490
	Suction trap (Hirst, 1952, 1953)						
Lycopodium	Efficiency (%)	87	87	71	54	55	—
	Detection threshold	2	2	2	3	3	—
Ustilago	Efficiency (%)	77	80	65	71	83	—
	Detection threshold	2	2	3	2	2	—

The obvious need for greater precision should not obscure the advantages of the method used. The freely exposed sticky-surface spore traps, most often used previously, did not estimate spore concentration, the value of nil records, or the hour-by-hour changes in spore concentration. Because the efficiency with which any trap catches ascospores (13 × 6 μ) or conidia (12–22 × 8 μ) of *V. inaequalis* is unknown, the accuracy of different traps cannot yet be directly compared. However, detection thresholds calculated from tests with larger and smaller spores in turbulent air in the wind tunnel at Rothamsted (Table 1) do allow freely exposed surface traps to be compared with suction traps. Gregory & Stedman (1953 and unpublished) showed that the efficiency of a 3 × 1 in. microscope slide inclined at 45° to the horizontal, with its sticky upper surface presented to the wind, varied less than when oriented vertically or horizontally. Such slides were tested with spores of *Lycopodium clavatum* 32 μ dia., *Erysiphe graminis* 24–30 × 12–15 μ and *Ustilago avenae* 6–7 μ diam. and their efficiency,

as a percentage of the 'area dose' was used to calculate detection threshold concentrations during an exposure of 1 hr. to winds ranging from 0·5 to 9·5 m./sec., assuming that 28 mm.^{2} of all deposits was scanned. Under these conditions the suction trap was tested only for *Lycopodium* and *Ustilago* (Hirst, 1953) but always had a lower and less variable detection threshold. The inclined slide is least accurate at low wind speeds but samples more air and catches spores more efficiently at high wind speeds. Spore size greatly affects the catches of the inclined slides whereas it is of little importance in the suction trap.

ASCOSPORES

Measurement of ascospore number

The apple orchards selected had electricity nearby and someone willing to service the instruments daily. Those where ascospore dispersal was measured were all commercial or experimental orchards where it was seldom possible for trees to be left unsprayed.

Ascospore trapping sites

Wisbech H (A. Hudson, Wisbech St Mary, Cambs.)
Duration: 1953–9.
Variety: Bramley's Seedling 40 years.
Ground cover: short grass.

Wisbech G (Garford and Co., Newton, Wisbech, Cambs.)
Duration: 1956, 1957.
Variety: Bramley's Seedling 50 years.
Ground cover: short grass.

Sudbury (Peter Wheldon Ltd., Sudbury, Suffolk.)
Duration: 1955–59.
Varieties: 2/3 Worcester Pearmain, 1/3 Laxton's Superb.
Ground cover: short grass.

East Malling (East Malling Research Station, Maidstone, Kent.)
Duration: 1956–9.
Variety: Bramley's Seedling.
Ground cover: experimental orchard, part short grass, part arable.
Acknowledgement: we are indebted to Dr R. T. Burchill for permission to publish counts which he made on the slides exposed in 1958 and 1959.

Long Ashton (The Research Station, Long Ashton, Bristol, Somerset.)
Duration: 1957.
Varieties: mixed cider.
Ground cover: short grass.

Evesham (G. A. Aldington, Offenham, Evesham, Worcs.)
Duration: 1956.
Varieties: Cox's Orange Pippin and Newton Wonder.
Ground cover: grass.

Traps were always placed as near the middle of the orchard as possible to decrease effects on spore concentrations from changes in wind direction. At each site a bimetallic thermograph, a hair hygrograph and a recording rain gauge were used to measure temperature, relative humidity and the time, duration and amount of rain. At Wisbech, Sudbury, Evesham and Long Ashton the instruments were within the orchards but at East Malling they were in the meteorological enclosure nearby. Records were also made, within orchards, of the duration of surface wetness (Hirst,

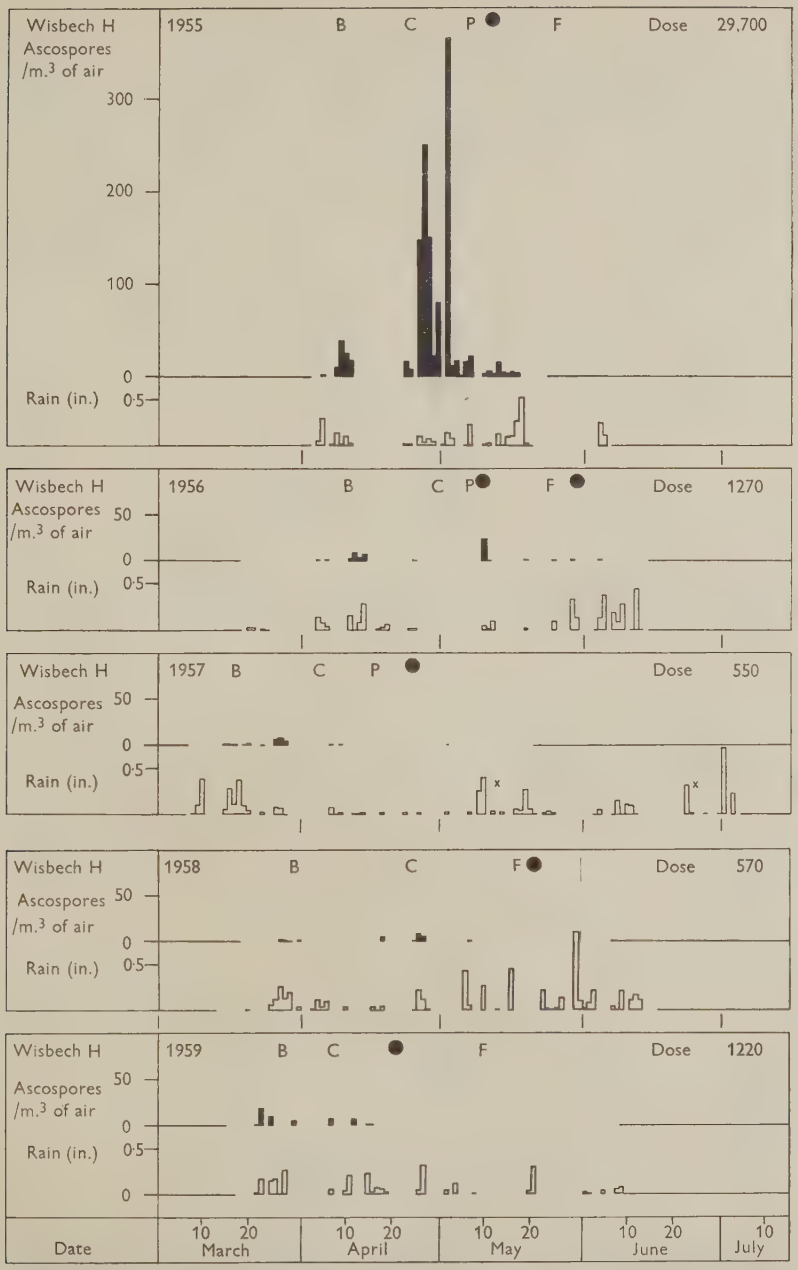

Fig. 1. Daily mean concentration of *Venturia inaequalis* ascospores in Wisbech H orchard related to rainfall, appearance of apple scab (●) and development of apple buds (B = bud-burst, C = green cluster, P = pink bud, F = petal fall). × in Figs. 1–4 indicates that a record was defective or lacking.

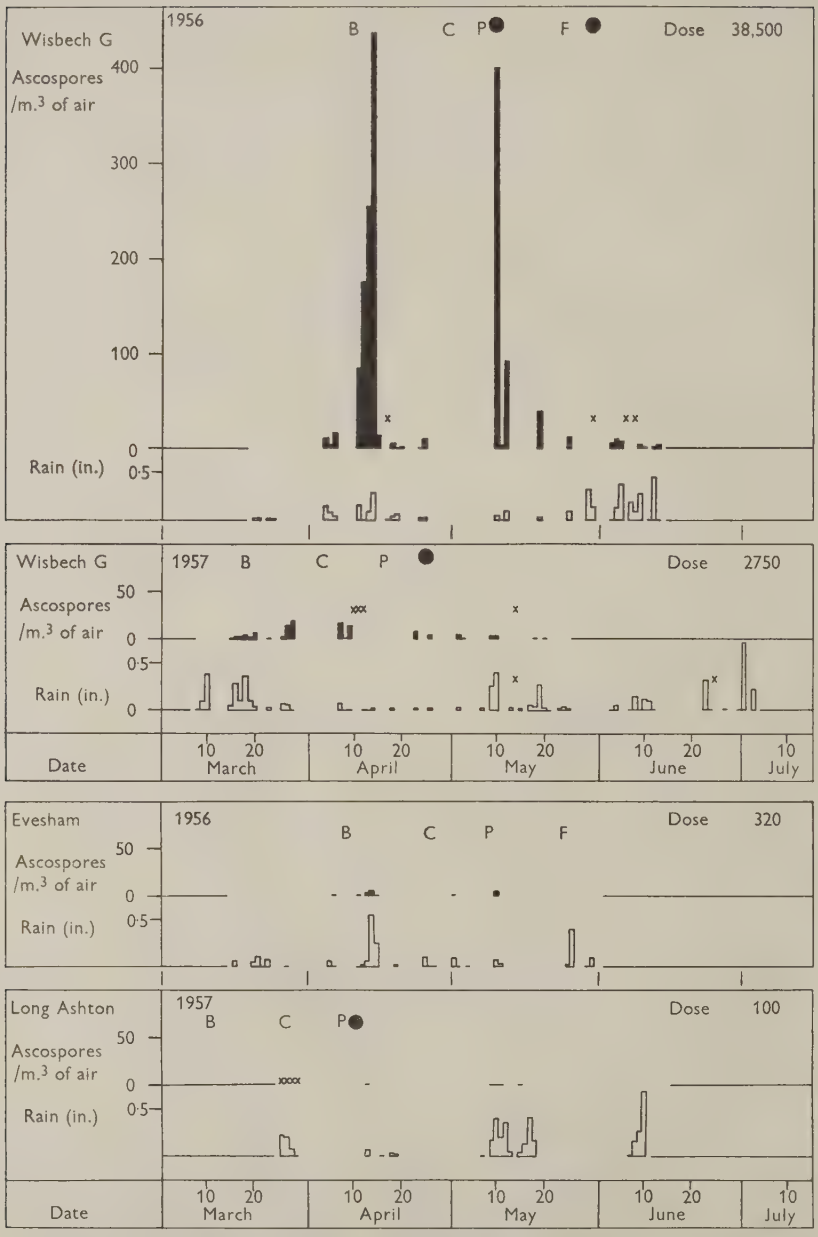

Fig. 2. Daily mean concentration of *Venturia inaequalis* ascospores and rainfall in Wisbech G, Evesham and Long Ashton orchards. Symbols as in Fig. 1.

1957) but in the present context are of interest only because they indicated the frequency of dews.

Observations in 1953 and 1954 (Hirst *et al.* 1955) supported an earlier conclusion that ascospores were common in air only during or soon after rain (Keitt & Jones,

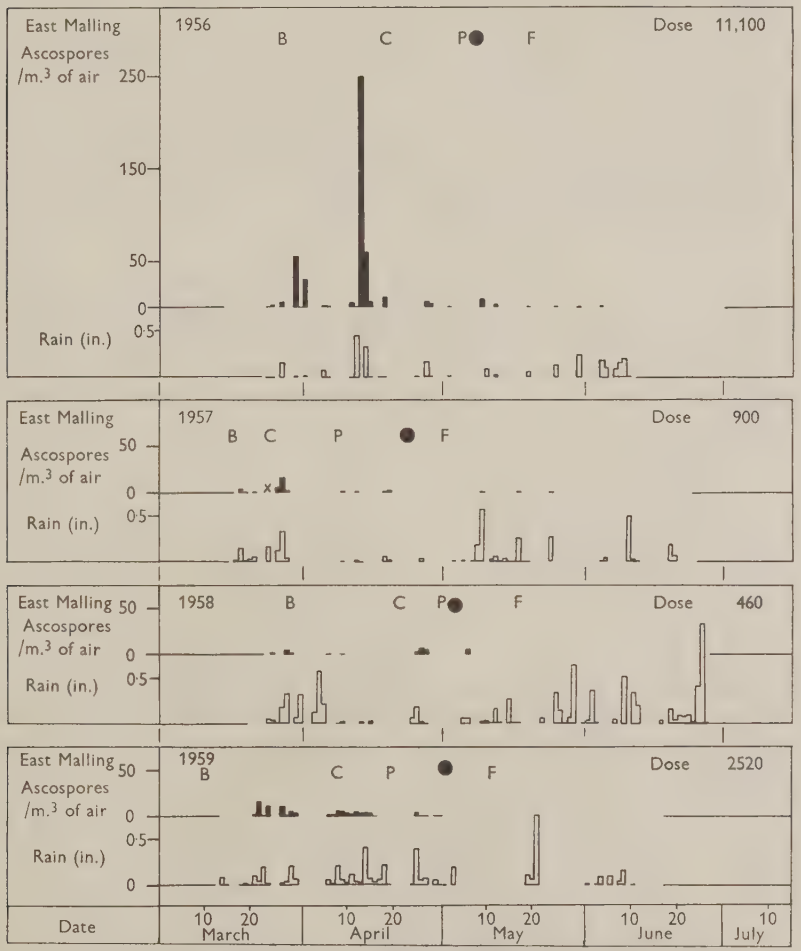

Fig. 3. Daily mean concentration of *Venturia inaequalis* ascospores and rainfall at East Malling. Symbols as in Fig. 1.

1926). In later years, only the catches during wet periods were examined, so that more orchards could be studied without greatly increasing the time occupied by scanning. The first traverses were made at least 1 hr. before the time when rain began to fall and continued until no *Venturia* spores were counted on at least three traverses

after rain ceased. The later records therefore omit any ascospores which may have been present on nights with dew but no rain. However, previous results suggested that these omissions would be unimportant. Strict standards of classification were

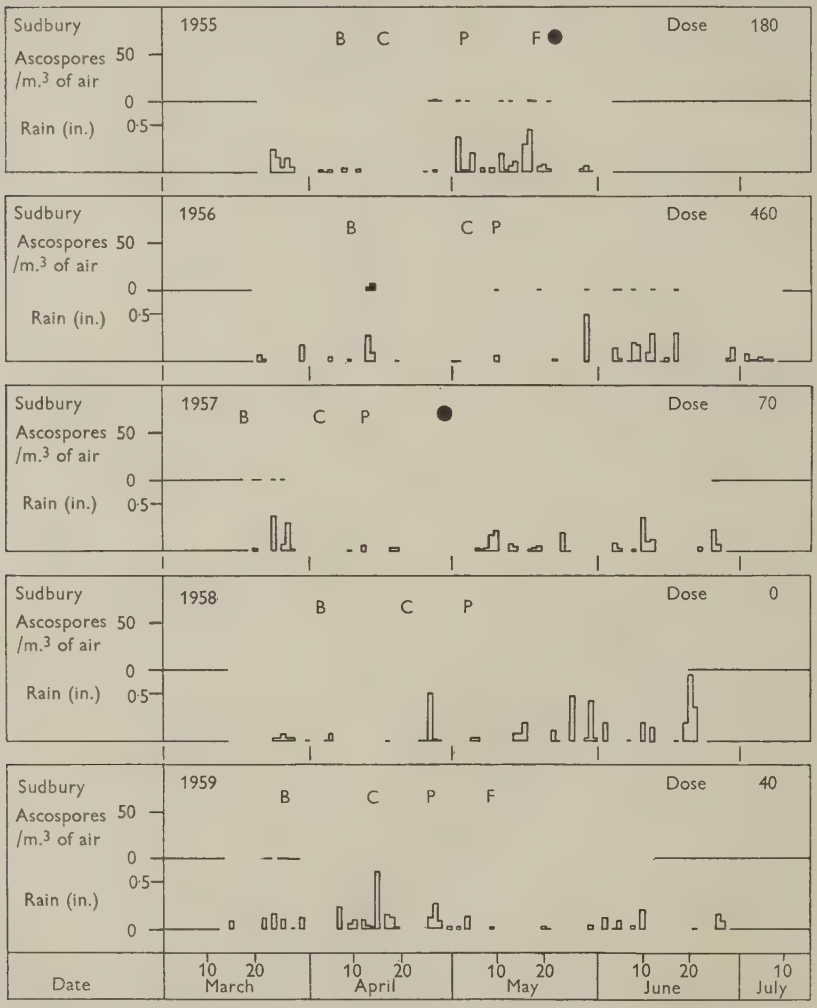

Fig. 4. Daily mean concentration of *Venturia inaequalis* ascospores and rainfall at Sudbury. Symbols as in Fig. 1.

adopted, as unidentified ascospores resembling those of *V. inaequalis* were common, usually in May and June. Many of the slides were re-counted to ensure that these others were excluded from the counts: this was possible because, although asymmetrically septate and released by rain, the unidentified spores differed from

V. inaequalis ascospores in being slightly smaller, more perfect ellipsoids and grey rather than greenish-brown. They were not among the spores caught when dead apple leaves were wetted in the laboratory, and so probably came from some other source.

In this paper we are concerned with the number of ascospores (or conidia) liberated and their phenology rather than with resulting infection. Daily mean concentrations serve this purpose and are shown in Figs. 1–4 with symbols indicating the dates of various growth stages and when the first scab lesions were found on leaves in the vicinity. The solid black histograms show the average concentration of ascospores in the 24 hr. prior to 09.00 G.M.T. on the dates for which they are plotted and the hollow histograms show the total rain during the same intervals. In these diagrams the customary line for the time ordinate is omitted for the period when the instruments were in use, to allow all days with rain or ascospores to be shown, since the finest reproducible line occasionally exaggerates the lowest amounts of either.

'*Relative ascospore dose*'

Like many other foliage pathogens, *V. inaequalis* inflicts damage by causing many local lesions. The extent to which it prospers in sprayed orchards depends both on the number of spores produced and the imperfections of the fungicidal control. To study how severity of the disease depends on ascospore number, it is useful to compare the number in one year or orchard with that in another. The hourly estimates of ascospore concentration for each orchard each year have been totalled; the figures have no absolute meaning but can be used to compare the annual dose of airborne ascospores to which trees around the trap are exposed. We have used this concept of 'relative ascospore dose' or 'dose' to illustrate how supply of inoculum varied (Fig. 5). The magnitude of changes in ascospore frequency shown in the diagrams is worth stressing. The heaviest dose was in Wisbech G in 1956, as was the highest concentration in any hour—just over 4000 ascospores per $m.^3$ of air. By contrast, no ascospores were found when scanning the slides from Sudbury in 1958. Within one orchard (Wisbech H) the highest dose was 54 times the lowest and in the two Bramley's Seedling orchards at Wisbech the dose differed by a factor of 30 times in 1956. High doses occurred in Bramley's Seedling orchards at Wisbech and East Malling up to 1956 but never at Sudbury. Wherever it was possible to judge, the dose declined between 1955 and 1957, remained low in 1958 and showed a slight increase in 1959. Unfortunately, there is insufficient information to justify any conclusions on the way in which dose may be influenced by the location of the orchard or the apple variety planted.

Phenology of ascospore liberation

It was impracticable to scan all slides daily but in the early years those for particularly important periods were examined soon after exposure. However, the results were used in analysing the success of spray programmes and developing generalizations about spore release, which were valuable as a guide for advice in later years. Spore-trapping usually started just before bud-burst and continued for 2–3 months until no ascospores were found on slides exposed on several consecutive rainy days. Microscopic

examination of perithecia occasionally showed that a proportion contained mature ascospores before the traps were erected. Precocious liberation might not have been detectable, and because ascospores would be unlikely to cause infection before the new leaf was exposed at bud-burst it should not be included in estimates of dose.

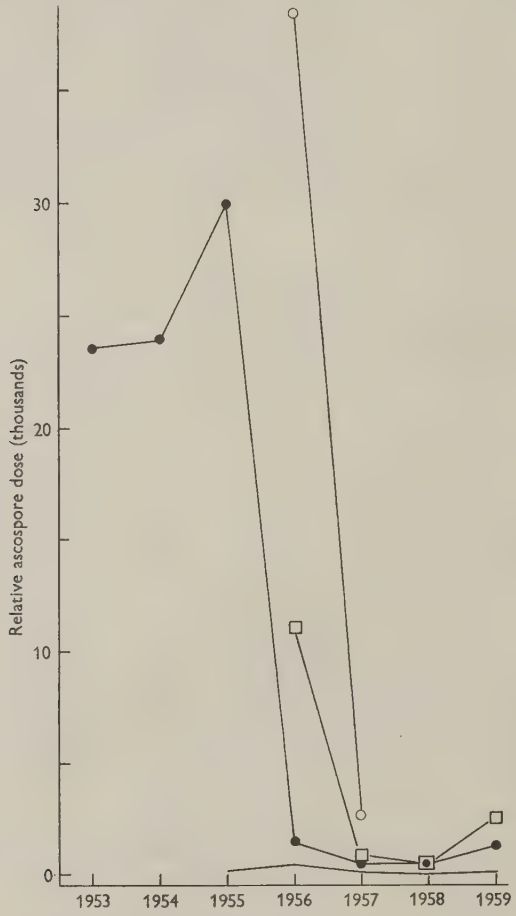

Fig. 5. Year-to-year changes of 'relative ascospore dose' in orchards in which spore traps were operated. —□—, East Malling; —●—, Wisbech H; —○—, Wisbech G; ——, Sudbury.

The seasonal pattern of liberation is much affected by the occurrence of rain. The highest concentrations usually accompanied rain which ended a dry spell of several days, during which ascospores had presumably matured but not been released. The season of liberation was extended by dry spells, as in 1956, and shortened by frequent rain, as in 1955. This effect is difficult to distinguish when the dose is low,

for then only the heavier liberations are detected, a point well illustrated by comparing Wisbech H and G in 1956 (Figs. 1, 2).

When infection has been prevented until up to the end of ascospore liberation the later part of the spray programme can often be relaxed without serious harm to the quality of the fruit. However, the date of the last discharge is difficult to fix as it is not regularly associated with any definite stage in the development of the trees and, particularly where the dose is low, may not be detected by traps. Even in orchards with high doses, where spores were detectable with almost every rain, ascospores were found on fewer than 30 days each season. Previous records of the days when ascospores were caught by traps or ejected on to sticky slides placed close above infected dead apple leaves show that this number is rarely exceeded. This suggests that, in all but exceptionally dry years, a safe and simple way to define the date when danger from ascospores ceases, is to count up to the thirtieth day with measurable rain following bud-burst. Where the dose was low the method would err on the side of caution, for example, in Wisbech H ascospores were detected on 23, 26 and 26 days in 1953 to 1955 but only on 14, 12, 9 and 7 days in the succeeding years.

CONIDIA

In the orchards where spore traps were erected in spring we found no conidial infections on twigs or on bud scales, although bud-scale infections have previously been reported on Bramley's Seedling at Wisbech (Dillon Weston, Storey & Ives, 1952). Without local sources, conidia could have started attacks only if they were dispersed over considerable distances, probably by air. In 1953 and 1954 when counts were made every 2 hr., and in the wet periods examined in later years, conidia were found only once before the disease appeared on new leaves (a clump of three conidia at East Malling, 09.00 G.M.T. 17 March 1957). In summer, traps were installed, if possible among heavily infected trees, to test whether conidia were air-dispersed and if so whether they could be caught efficiently.

Catches on slides from these orchards were examined regularly at 1- or 2-hr. intervals on both wet and dry days but otherwise the scanning procedure was the same as for ascospores.

Conidial trapping series

1953. Harpenden A.
Orchard in garden adjoining Rothamsted Experimental Station (R.E.S.).
Duration: 4 to 28 June.
Records: Temp., R.H., sun, rain; all at R.E.S.
Summary: Only 22 of the 288 2-hourly counts showed conidia, 20 of them were between 09.00 and 21.00 G.M.T. and 15 in dry sunny periods. The highest concentration estimated was 70 conidia per m.^{3} of air.

1953. Harpenden B.
Garden half a mile north-east of R.E.S. meteorological station.
Duration: 3 to 27 July.
Records: Temp., R.H., radiation, sun, rain: all at R.E.S.
Counts: 2-hourly at odd hours.

1954. Wisbech H.
Bramley's Seedling, sprayed commercial orchard.
Duration: 7 June to 9 September.

1954. Wisbech H. (*cont.*)
Records: Temp., R.H., rain for part of period.
Summary: Conidia were detected on 29 of the 73 days but in only 52 of 876 hourly estimates (42 of them between 08·00 and 20·00 G.M.T.).
Acknowledgement: We are grateful to Dr I. F. Storey and Miss J. V. Ives who made these counts.

1954. Tewin (Herts.).
Small commercial orchard of mixed dessert varieties 10 miles E.S.E. of R.E.S.
Duration: 29 June to 28 July.
Records: Temp., R.H. and rain on site. Sun and radiation from R.E.S. only.
Counts: 2-hourly at odd hours.

1956. Harpenden C.
Small orchard of mixed varieties ½ mile west of R.E.S. meteorological station. Sheltered by woods.
Duration: 23 July to 4 September.
Records: Temp., R.H., sun, radiation, rain, wind run; all at R.E.S.
Counts: Hourly.

Table 2. *Occurrence and concentration of* Venturia inaequalis *conidia in three trapping series in relation to sunshine and rain*

	Intervals without sun or rain	Intervals with sun	Intervals with rain	Intervals with rain and sun
	Harpenden B, 1953			
2 hr. intervals without conidia	84	44	14	3
2 hr. intervals with conidia	43	93	14	18
Mean conc. of conidia when present (no. per m.^{3} of air)	45	154	44	103
	Tewin, 1954			
2 hr. intervals without conidia	82	46	31	2
2 hr. intervals with conidia	56	89	16	8
Mean conc. of conidia when present (no. per m.^{3} air)	24	72	31	113
	Harpenden C, 1956			
Hourly intervals without conidia	594	210	151	22
Hourly intervals with conidia	39	98	10	10
Mean conc. of conidia when present (no. per m.^{3} of air)	44	65	39	53

The results of all series showed that airborne conidia were most common in daytime and in dry weather although they were also caught during rain. When they occurred in clumps the conidia were often shrunken, dirty and a darker brown than single conidia. Two orchards, Harpenden A, 1953 and Wisbech H, 1954 (which was among sprayed trees) yielded few conidia and have not been included in the analyses (Table 2) but their results are summarized above. To illustrate features common to all orchards, detailed records for part of Harpenden B, 1953 are reproduced in Fig. 6. The first 4 days of July (not shown in Fig. 6) were cool and dull and there were never more than 25 conidia per m.^{3} of air but then the maximum concentration increased daily until it reached 1320 conidia per m.^{3} at 17.00 G.M.T. on 8 July. During this time rain fell only as light showers early in the mornings of 3 and 6 July. In these and the later days shown in Fig. 6 conidia were common only in the daytime, particularly

during sunshine with low humidity. Rain by day usually lowered the concentration, unless the 2-hr. interval was also sunny, but at night when conidia were usually absent there was occasionally evidence of a small liberation accompanying rain.

This characteristic behaviour relative to weather and time of day applies throughout the three series of slides with highest catches, which are analysed in Fig. 7. The mean concentration of conidia throughout each series was 61 per m.^{3} of air in Harpenden B, 1953; 28 in Tewin, 1954; 8 in Harpenden C, 1956; and conidia were detected during 54, 51 and 14%, respectively, of the intervals sampled. In all series

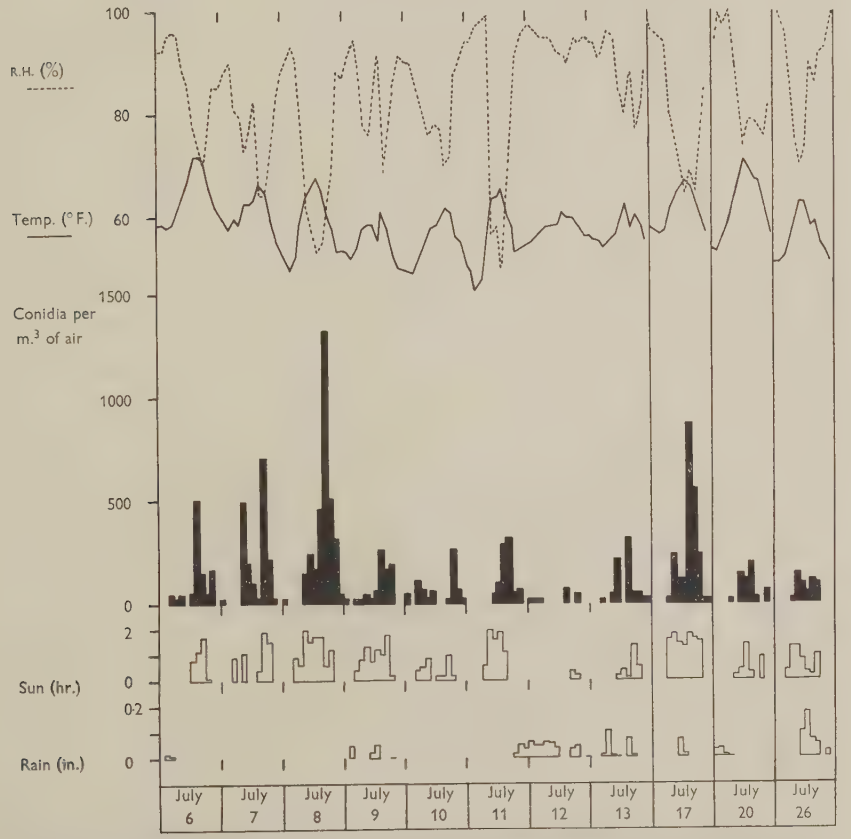

Fig. 6. 2-hourly estimates of the concentration of *Venturia inaequalis* conidia in the air of Harpenden B orchard in 1953, related to sun, rain, temperature and relative humidity.

the highest concentrations were during intervals with sunshine; but in dull weather or in the dark, rain seemed not to affect the proportion of intervals with conidia nor their mean concentration. It is not surprising, therefore, that concentrations of *V. inaequalis* conidia are greatest in daytime. The curves in Fig. 7 are calculated

from geometric means of spore concentration at each time of day and expressed as a percentage of the highest (see Hirst, 1953). The three series agree in general but there are interesting differences in detail. Harpenden B, 1953 showed the largest range and both it and Tewin, 1954 were strongly bimodal, although the subsidiary peak occurred in the afternoon at Harpenden and in the evening at Tewin. In Harpenden C, 1956 the peak concentrations persisted for a shorter time and there was little sign of a subsidiary peak. The reasons for these differences are not known but may be connected with the fact that the small orchard used for Harpenden C, 1956 was sheltered by tall trees except to the south.

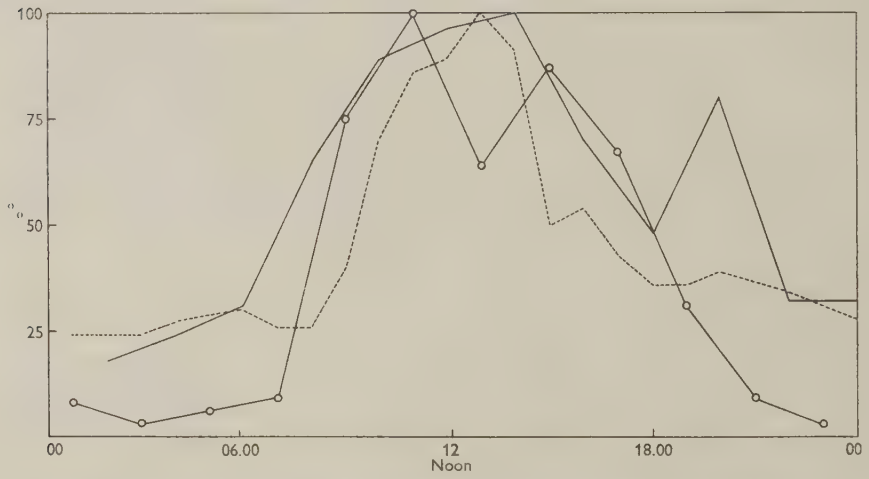

Fig. 7. Diurnal periodicity of *Venturia inaequalis* conidia expressed as percentage of the peak geometric mean concentration. Harpenden B, 1953, O—O; Tewin, 1954, ——; Harpenden C, 1956 – – – –.

DISCUSSION

To justify the conclusion that ascospores are the chief cause of first infection in orchards lacking overwintering conidial pustules, it was necessary to demonstrate that our traps could catch airborne conidia and that in spring these were rare or lacking. Few have disagreed with the opinion of Frey & Keitt (1925) that 'no important dissemination of conidia is to be expected in the absence of water, though undoubtedly some spores are dislodged by wind whipping of leaves, fruit or branches, by contact with wind-blown particles and in other minor ways. It therefore appears that the important agency for dissemination of these conidia is meteoric water moving under the influence of wind and gravitation.' Conidia are unquestionably detached by wetting, so that splashed or dripping water probably does constitute the main dispersal mechanism within trees. Some splash-dispersed conidia may travel long distances in air when the droplets containing them evaporate before deposition, but these seem inadequate to explain the frequency with which the disease becomes established in summer once an effective spray programme has ended. Most authors

have dismissed the possibility that these infections might result from airborne conidia because they caught few except on wet and windy days and could not show that they were detached by even strong jets of air. The detection thresholds for freely exposed inclined microscope slides quoted in Table 1 suggest that they would seldom detect low concentrations such as we have found persisting for long periods in fine weather. Even when a few conidia were caught on slides exposed for 24 hr., in unsettled weather it would be impossible to decide whether they were deposited dry or trapped preferentially while included in relatively heavy splash droplets. Furthermore, in high winds, freely exposed slides sample more air, more efficiently, so it is not surprising that most conidia were caught on wet and windy days. It is much more difficult to explain why conidia were not detected in dry weather with the earlier suction traps (Frey & Keitt, 1925; Keitt & Jones, 1926; Wiesmann, 1935). Unless sited among heavily infected trees the concentration of conidia is usually small and the catch may have been decreased by imperfect collection at sampling orifices, which were not directed into the wind and had only a low intake velocity. The paper or nitro-cellulose filters may have had a higher retention efficiency in wet than in dry weather or in the rigorous preparation for counting the conidia may have become so shrunken that they could not, with certainty, be distinguished from similarly coloured spores, e.g. *Cladosporium*.

Our traps were operated close to infected trees to ensure maximum concentrations, but even so there is no reason to doubt that a small proportion of the conidia could travel long distances to other orchards. (In fact *V. inaequalis* conidia have recently been caught by spore traps in aircraft flying at 2000 ft. over the middle of the English Channel.) We did not measure the viability of conidia collected from orchard air but laboratory experiments (Wallace, 1913; Keitt & Jones, 1926) suggest that conidia resist desiccation. However, a very few viable airborne conidia could be important epidemiologically if they established the disease in a clean orchard after spraying has ceased. Then, in favourable weather, the fungus can multiply rapidly on the tip leaves of extension growths and ensure ascospores in plenty the following spring.

Ascospores are ejected into the air after rain, but measurable concentrations are generally short-lived. By contrast, conidia seem to persist in small numbers through many of the daylight hours during a period of up to 6 months. The threat of infection is therefore 'chronic' but real, providing viability is not very quickly lost. Comparison of hourly estimates of spore concentration with continuous weather records often provides clues to the way in which various types of fungal conidia are liberated, but often, as here, there are too many interacting variables for any definite conclusions to be reached.

The occurrence of many ascospores in spring has been amply confirmed. Unfortunately, trapping at different heights prevented any direct comparison between the highest concentration we measured (4000 ascospores per $m.^3$ of air, 2 m. above ground, Wisbech G, 1956) and the catch (of 21,600 per $m.^3$ 0·5 m. above ground) reported by Miller & Waggoner (1958).

In unsprayed orchards, assuming equally favourable weather and hosts, initial infection would be proportional to spore concentration, but severe epidemics can develop from a few initial infections resulting from small ascospore concentrations. In orchards where the sprays are well timed, most spores are killed by fungicides.

The chance of infection then depends on the probability of spores being deposited where fungicidal cover is defective. High ascospore doses therefore increase not only the potential infection, but also the precautions necessary to check the establishment of the disease.

Ascospore dose is certainly determined by many factors, which include cultural and control practices, the apple variety, the amount and date of leaf infection and the amount of dead leaves present in spring. It seems reasonable to proceed on the assumption that one of the most important factors is the proportion of leaves infected by the previous autumn. Thus, low doses would follow dry years when few opportunities for infection occurred and chemical control was good. Only in Wisbech H have we records extensive enough to suggest that dose declined from a sustained high level to a low one. Dose decreased in both Wisbech H between 1955 and 1956 and in Wisbech G between 1956 and 1957 when lime-sulphur sprays were abandoned in favour of phenyl mercury compounds timed for post-infection or curative action (see Storey & Ives, 1956). However, it would be unwise to assume these happenings to be cause and effect, because the decline in Wisbech G was paralleled at East Malling, where the protective lime-sulphur programme remained unchanged. The weather may have caused all the decreases but its influence could not be measured in sprayed orchards. It seems more likely that the decreases resulted from both weather unfavourable to the fungus and spray programmes adequate for this weather. Had we been able to study unsprayed orchards, the results, although valuable, would have been mainly of academic importance, for growers are inevitably concerned with the interaction between host, parasite and fungicide.

The observations do not permit the doses in orchards of different varieties to be compared. Although high doses were found only in Bramley's Seedling orchards, this does not necessarily mean they were a superior substrate for perithecial development, because dose must be influenced by the amount of dead leaf present in spring and this varied considerably between orchards and seasons.

We have used the concept of 'relative ascospore dose' for comparing inoculum level between orchards and seasons. This provides an index of the total concentration of airborne ascospores to which leaves 2 m. above ground were exposed each season after the first signs of bud-breaking. The concept is certain to need refinement in the future because it has several important limitations. For example, the size of the orchard and proximity of others probably influence the number of spores caught, as do the distribution of wet and dry spells, the duration of rainfall, the weather at the time of liberation and control and cultural practices. None of these factors invalidates dose as defined, but all emphasize that it is neither a valid measure of the full potentiality of the orchard to produce ascospores, nor an indication of the danger of infection. Ascospores are often released in weather not conducive to infection and these will be wasted unless they can survive until infection conditions recur. The 'effective dose', an estimate of the proportion of ascospores able to succeed, would therefore be a more valuable measure and one we should aim to make.

These observations could not have been made without the generous provision of orchard facilities and willing help in maintaining our apparatus day by day. We

wish to thank the growers mentioned and the Directors and plant pathologists of East Malling Research Station and the Research Station, Long Ashton. We are also indebted to the Director General and Mr L. P. Smith of the Meteorological Office for the loan of apparatus and for advice.

We cannot mention all those who have helped but hope that they will excuse our naming only those associated with the work for the longest periods: Mrs J. V. Baker (Miss J. V. Ives) and Dr I. F. Storey from the N.A.A.S. Provincial Headquarters at Cambridge, Mr R. Souter at East Malling, Mr F. Jacobs at Sudbury and the invaluable Mr W. C. Ward at Wisbech. Miss Maureen Thomson of Rothamsted helped to count the slides and to prepare the diagrams.

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The influence of sunshine and rain on tea blister blight, *Exobasidium vexans* Massee, in Ceylon

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SUMMARY

The germination of spores of blister blight of tea (*Exobasidium vexans* Massee) requires moisture, but 0·1 in. of rain in a day provides sufficient for a high percentage of germination to occur; more rain does not materially increase germination. Spores are killed by sunshine of a few hours' duration, even if there is adequate rain on the same day. An average of about $3\frac{3}{4}$ hr. of sunshine per day over 5 days is enough to reduce the blister-blight disease to subeconomic levels. Ten-day spraying rounds with copper fungicide usually give adequate control of the disease but add to the cost of production. A method is put forward of using recorded sunshine hours as the criterion in deciding on the frequency of spraying; spraying is postponed, by successive 5-day periods, until the average sunshine for the previous 5 days has dropped below $3\frac{3}{4}$ hr. A reduction approaching one-half of the number of spray applications can be made in this way.

INTRODUCTION

Blister blight of tea has been known from Assam for a long time (Massee, 1898) but it did not appear in Ceylon until October 1946 (Tubbs, 1946). It then spread so fast as to threaten to destroy the tea industry in mid-country and up-country districts, but a remedy was quickly found in routine spraying with a copper fungicide. This spraying is now effective but makes a not inconsiderable addition to cost of production, so various attempts have been made to reduce the expense. The work here described provides a basis for making savings, by omitting to spray when the weather conditions indicate that it will be unnecessary. Practical field tests on commercial estates have since demonstrated the value of the method (Mulder, 1959; Mulder & De Silva, 1960).

Gadd & Loos (1949) showed that wind-borne spores germinate on the leaf in humid conditions, the leaf surface is penetrated and, after a week or two, a dense growth of hyphae inside becomes visible as a translucent spot. Further growth presses out and eventually bursts a blister on the underside of the leaf. Spores are produced there and discharged for about a week. The fungus then dies, leaving a damaged or perforated leaf. Only the youngest leaves and their stems are vulnerable but it is these that provide the tea crop. The disease not only reduces the crop but may also so limit the active foliage as to weaken the bush.

The disease is very active only in the wetter parts of the year, when fungicidal sprays are normally applied every 5–10 days, to fit in with the plucking rounds. It is

not therefore surprising that the need for spraying is commonly associated with the incidence of rain, dew, or mist; in fact a system of control based on the average relative humidity has been recommended previously (Huysmans, 1952). On the other hand, several workers have suspected or investigated the importance of sunshine (Gadd & Loos, 1949; Huysmans, 1952; Homburg, 1953, 1955; Van der Knaap, 1955; De Weille, 1957*a*, *b*) and suggested that control measures could be relaxed in sunny periods. These workers have shown that exposure to direct sunshine for ½–1 hr. is sufficient to kill the spores. As rain and sunshine are obviously inversely correlated to some extent, careful work is required to separate their effects. The leaf must necessarily be wet with rain, mist, or dew for most of the time during the monsoon; the question is: how much sunshine is required at this period to suppress the disease? Both Van der Knaap (1955) and De Weille (1959) introduced control systems based on sunshine, but both systems require a specific loading of sunshine hours according to time of day or number of hours of sunshine in the day and are therefore not simple enough for routine use on estates.

SPORE GERMINATION

As it is mainly the spores that are affected by weather, they were investigated first. Spores were collected in the laboratory on glass slides kept for a few hours under detached tea shoots with sporulating blisters (Wolthuis, 1958). Pairs of these slides carrying the spores were then placed in a tea plantation in slits cut at the tops of 5-ft. sticks; usually five such sticks were stuck into the ground about 5 p.m. amongst the tea, with the slides at the height of the plucking table. After 24 hr. the percentage germination of the spores was assessed from five random counts of twenty spores per slide on ten slides. Under favourable conditions in the field, spores formed germ tubes two to four times the length of the spore in that time, but the appressorium stage was seldom reached. Weather observations were made both for the first day, when the slides were put out, and for the second, when they were taken in.

Rate of germination as observed on glass slides is not necessarily the same as that on leaves, e.g. the age of the leaf has an effect on germination (Loos, 1951). However, it was found by Wolthuis (1958) and confirmed by ourselves that the germination as observed on glass slides gives a good qualitative indication of conditions in the field.

Spore germination percentages recorded daily (I) in May–June 1958 (thirty-five observations) in a shaded field and (II) in June–July (fifty observations) in an unshaded field were correlated with daily sunshine and rainfall; (III) the average germination percentage over twenty-nine consecutive 5-day periods between June and October 1958 was correlated with average sun and rain over the same 5-day periods. There was a significant inverse correlation ($-r = 0{\cdot}44$–$0{\cdot}60$) between sunshine and rainfall. Consequently, it is not surprising that the correlation between spore germination and sunshine ($-r = 0{\cdot}46$–$0{\cdot}71$) on the one hand and between germination and rainfall ($+r = 0{\cdot}35$–$0{\cdot}66$) on the other were significant in both instances.

These correlations did not suggest that either sunshine or rainfall had the greater importance. Figs. 1 and 2 represent all the data in a summarized form (averages of

consecutive classes of daily rainfall or sunshine) showing (average) germination as a function of (average) rain or sun.

In Fig. 1 is shown the relation between germination and rainfall. There was a clear relationship up to 0·1 or possibly 0·2 in. of rain per day, but above 0·25 in., the percentage of germination hardly depended on rain.

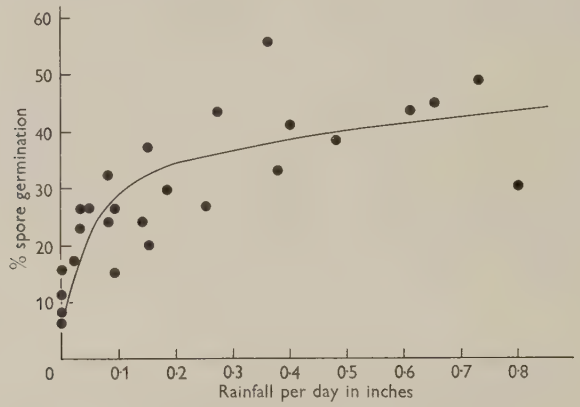

Fig. 1. Relationship between germination of spores of blister blight and daily rainfall.

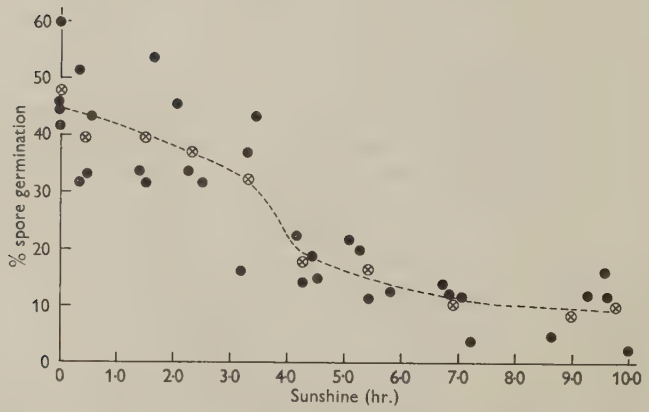

Fig. 2. Relationship between germination of spores of blister blight and duration of sunshine in the day; black dots are observations, crossed circles are averages per sunshine category.

Fig. 2 shows the relation between germination and sunshine. With up to 3 hr. of sunshine in the day, germination was always high but with 4 hr. or more, it was low. Such relations are not expected to give a large value of correlation coefficient.

Fig. 3 therefore re-classifies the observations on germination; in Fig. 3*a*, where germination is shown against hours of sunshine, observations from days with less than

0·1 in. rain are shown as solid columns, and those with 0·1 in. or over in cross-hatched columns. With 6 hr. or more of sunshine, the amount of rain was immaterial and indeed it was not very important unless there had been 2 hr. or less of sunshine. In the same way, from Fig. 3*b*, it can be seen that with less than 4 hr. of sunshine, germination was high, especially with more than 0·1 in. rain; but with more than 4 hr. of sunshine it was almost independent of rainfall. For practical purposes, therefore, it would seem that 4 hr. of sunshine, or a little less, is a threshold, providing the simplest single measurement as a criterion in those situations where the position cannot be estimated by eye (i.e. some rain and some sunshine). These observations provide indirect confirmation of the results of De Weille (1957*a*) on the direct action of sunshine on spore viability.

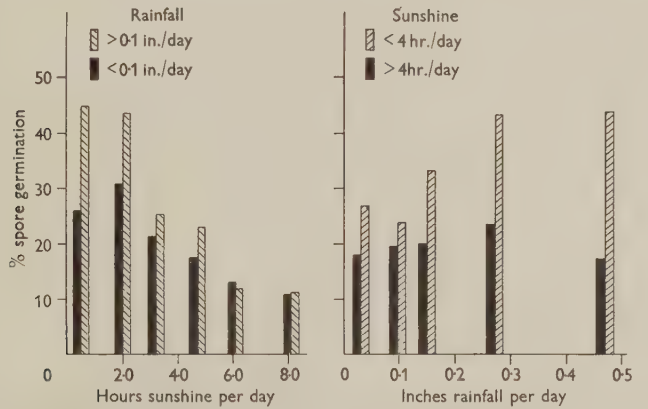

Fig. 3. (*a*) Average percentage of spore germination of blister blight in relation to sunshine: black columns—with rain less than 0·1 in. in the day; shaded columns—with rain more than 0·1 in. in the day. (*b*) Average percentage of spore germination in relation to rain: black columns—with more than 4 hr. sunshine in the day; shaded columns—with less than 4 hr. sunshine in the day.

BLISTER BLIGHT INCIDENCE AND WEATHER

The situation dealt with in the germination experiments was too simple for every-day practice, so correlations were sought between (*a*) translucent spot and (*b*) blister incidence on the one hand and (1) spore germination, (2) sunshine, and (3) rainfall occurring various numbers of days before (*a*) and (*b*) on the other hand. The incidence of translucent spots or blisters is defined as the numbers present per 100 leaves from crop shoots taken at random in the field. All the observations were made daily (I) both in a field with normal number of shade trees (May–June, 1958) and (II) also where there was no shade (June–July, 1958) during the south-west monsoon rains.

Fig. 4 shows that the correlation between percentage germination and incidence of translucent spots was at a maximum when the interval between them was about 12 days, whereas the maximum correlation with blisters was at 16 days. These times were unaffected by the presence of shade. They suggest the occurrence of correlations

of the incidence of the disease with the weather (rainfall and sunshine) of 12 days (translucent spot) and 16 days (blister) earlier. In fact, the highest correlation coefficient of spot incidence and weather occurred with an interval of 10 days with both sunshine (−0·67) and rain (+0·25) in the shaded field and of 13 days in the unshaded field, again with both sunshine (−0·48) and rain (+0·71). Blister incidence correlated best with the weather 16–17 days earlier. Again, there was a higher rainfall correlation without shade (+0·80) than with (+0·37) and a higher sunshine correlation (−0·56) when shade interfered than when there was none (−0·32). In both this instance and in the case of spot incidence, the infection appeared to have been affected most by sunshine in the presence of shade and by rain in the absence of shade, presumably because shade interferes with the drying of leaves.

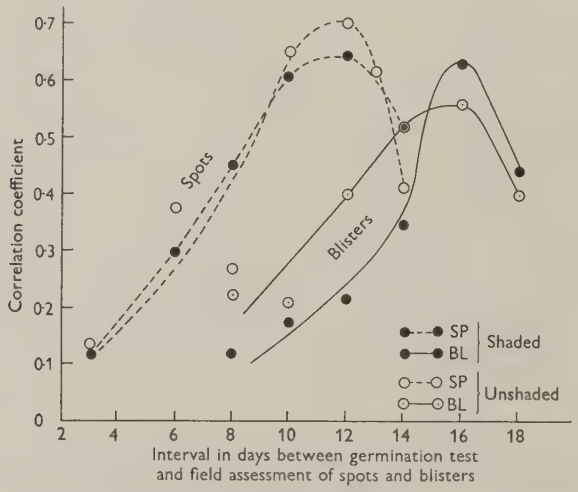

Fig. 4. Correlations between percentage of spore germination of blister blight on the one hand and incidence of translucent spot (SP) and blisters (BL) on the other, at various intervals (days) between germination test and field assessment of spots and blisters.

The period of 10–13 days required for translucent spot development and 16–17 days for blister development found on the basis of calculation are in fair agreement with direct observations by Huysmans (1952) and Loos (1951).

A further assessment made was of percentage shoot infestation by the standard method; this necessitates a careful system of random sampling of crop shoots to find the percentage of shoots that have the third leaf infected by visible blisters or translucent spots. This sampling was done at 5-day intervals for $4\frac{1}{2}$ months (June to October 1958) both in a shaded field (III) and in an unshaded one (II). Sunshine hours and inches of rain were totalled over 5-day periods and correlations with the incidence of the disease worked out. Maximal correlation existed with sunshine (−0·45 and −0·69) recorded 18–22 days and with rainfall (+0·42 and +0·69) recorded 16–20 days before shoot infection (twenty-nine averages).

The interval between weather and shoot infection was certainly longer than the interval for maximal correlation in the previous observations. This follows from an observation by Tubbs (1947), namely, that spores on the first or youngest leaf germinate in much less time than those on the older third leaf.

BLISTER BLIGHT AND SHADE

It is well known that the natural incidence of blister blight is much higher in shaded tea than in unshaded and indeed the first measure of control was to reduce shade. This is readily explicable as effects of both differences in sunshine and in dampness on

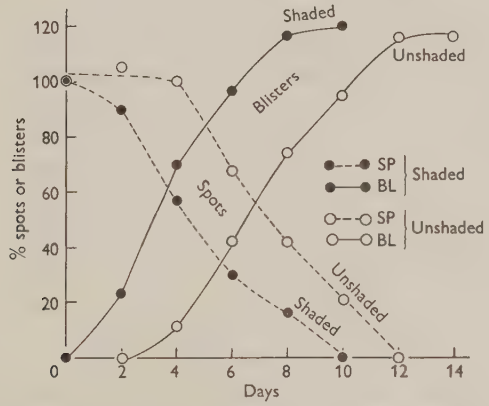

Fig. 5. The percentages of translucent spots (SP) of blister blight remaining as such after various periods in days (broken lines) and the percentages converted to blisters (BL). Some spots were initially missed, giving eventually over 100 % of blisters.

spore germination. What has not previously been recorded is that the weather also affects the development of the fungus at a stage as late as from translucent spot to blister. Two shaded bushes and two unshaded bushes were chosen in the same area and, on each bush, two shoots with three leaves were marked. They were observed every second day, the numbers of translucent spots and of blisters being counted. It is evident (Fig. 5) that the spots developed into blisters about 4 days more quickly under shade than without shade trees.

In this context Huysmans's (1952) observations may be mentioned; he found that the weather conditions prevailing during the period of blister development also have an affect on the germination rate of the spores subsequently released. Accordingly, the shading density of a field influences both the level and rate of infection.

CONTROL MEASURES IN RELATION TO SPORE GERMINATION

The planning of a practical control programme on the basis of the above information was done in two fairly direct stages without detailed working out of the course of events—namely using, first, spore germination and, second, recorded sunshine, to

determine whether to spray or not. The spore-germination procedure was taken from Wolthuis (1958), who determined daily the percentage germination on slides. There were four treatments:

(1) unsprayed control;
(2) sprayed when germination exceeded 50%;
(3) sprayed when germination exceeded 25%;
(4) sprayed on normal routine every 9–10 days.

The treatments were replicated five times in shaded plots of 0·1 acre and unreplicated in an unshaded field (0·25 acre per plot). The percentage infection of crop shoots was determined at 5-day intervals, thirty-five times in the shaded field (May–October 1958) and twenty-nine times in the unshaded one (June–October 1958).

Table 1. *Average monthly shoot infection of plots treated according to the degree of spore germination in the field*

The significant difference for means is 8·0 at $P = 0{\cdot}05$ (shaded field).

Treatments	Shoot infection (%) May	June	July	Aug.	Sept.	Oct.	Mean	No. of sprays
Shaded field (spraying was begun on 20 May in the sprayed control)								
(1) Control (unsprayed)	47·2	41·2	52·7	31·0	53·0	26·6	42·0	0
(2) Spore germ. > 50%	54·6	45·7	29·6	14·4	35·0	24·6	34·0	10
(3) Spore germ. > 25%	37·8	40·4	26·9	6·9	21·9	12·8	24·5	16
(4) Control (sprayed)	53·6	41·6	28·0	9·3	23·0	13·9	28·2	17
Unshaded field								
(1) Control (unsprayed)		8·7	19·6	15·3	18·2	10·1	14·4	0
(2) Spore germ. > 50%		4·4	12·6	3·1	10·9	6·5	7·5	6
(3) Spore germ. > 25%		4·3	7·4	2·6	3·2	2·0	3·9	13
(4) Control (sprayed)		8·3	11·1	4·7	3·3	2·3	5·9	14

In the experimental treatments, spraying was never done after a shorter interval than 10 days. In the 50%-germination plots, germination in the first 5 days after spraying was ignored. If, in the next 5 days, germination exceeded 50% on any one day, the spraying was done on the 10th day; but if it did not, spraying was postponed until the first day on which a 50% germination was observed. That began a new cycle, with germination ignored in the first 5 days. In the 25%-germination plots the routine was the same except that the criterion was 25% instead of 50%. The spray was high-volume, with a 50% Cu fungicide at 6 oz. per acre.

Table 1 shows the results. In the unshaded field, infection never reached economically damaging levels (35% infection—Webster & Park, 1956; Visser, Shanmuganathan & Sabanayagam, 1958) and it is evident that fourteen spraying rounds could have been saved. In the shaded field, infection in the 25%-germination plots did not differ from that in the normally sprayed plots, but only one spraying round was saved. In the 50%-germination plots, infection was always higher than in the normally sprayed plots, though protection was on the whole not unsatisfactory, and it saved seven spraying rounds. These observations confirmed the favourable results obtained by Wolthuis (1958). The idea was worth following up; but percentage germination of spores was not a suitable measure for routine work on estates.

CONTROL MEASURES IN RELATION TO SUNSHINE

The basis of decision in this experiment (previously summarized by Visser *et al.* 1959) was similar to that in the previous trials, except that the number of hours of sunshine in 5 days provided the criterion for one set and in 7 days for the other. Considering the 5-day set and the criterion of an average of 3 hr. sunshine per day, spraying was not done after 10 days if the total sunshine in the previous 5 days exceeded 15 hr.; spraying was withheld by 5-day periods until the previous 5 days' total fell below 15 hr. The treatments were randomized and replicated six times (0·1 acre each plot), with the two sets (5- and 7-day totals), and, in each set, averages of $2\frac{1}{2}$, 3 and $3\frac{3}{4}$ hr. per day used as the criterion (Table 2).

Table 2. *Average monthly shoot infection of plots sprayed according to the amount of sunshine recorded over 5- and 7-day periods, respectively*

	2	3	4	5	6	7	8	9	10	11
	Average monthly shoot infection (%)									Total no. of sprays
Treatments	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Mean	
(A) Control (sprayed)	14·0	26·0	28·5	7·7	22·1	10·7	17·1	8·3	*16·8*	24
(B) Control (unsprayed)	15·9	43·4	54·8	19·3	48·7	37·8	39·1	32·5	36·2	0
Sun/5 days										
(C) < $12\frac{1}{2}$ hr.	14·5	42·2	45·2	16·0	24·7	28·1	20·9	20·1	26·5	11
(D) $12\frac{1}{2}$–15 hr.	13·8	37·2	39·4	17·4	23·5	23·5	15·0	17·5	25·5	11
(E) 15–$18\frac{3}{4}$ hr.	15·3	33·8	26·0	12·3	16·5	18·1	15·9	19·5	*19·7*	13
Sun/7 days										
(F) < $17\frac{1}{2}$ hr.	18·4	47·7	42·9	17·7	14·1	27·7	23·3	25·9	27·2	9
(G) $17\frac{1}{2}$–21 hr.	17·5	46·3	39·0	14·6	12·8	21·2	21·8	33·4	25·8	11
(H) 21–$26\frac{1}{4}$ hr.	15·9	45·3	34·8	12·7	17·7	19·5	18·6	12·6	*22·1*	17
Sign. diff. for $P = 0·05$	10·6	13·8	7·4	5·5	10·0	6·2	11·0	8·8	5·9	

The unsprayed plots were infected to damaging levels in all months except May, August and December (marginal). The normally sprayed plots (twenty-four spray rounds) were not badly infected at any time. The six series of experimental plots were generally more heavily infected than the normally sprayed plots, but up to economic levels (35% or higher) only in June and July (with the exception of the best treatment—E). Between seven and thirteen spray rounds were saved. Evidently the critical figures are those in June and July. Although the differences are not generally significant in pairs, there is a strong suggestion that the highest average used ($3\frac{3}{4}$ hr.) is marginal and that a 5-day total is more satisfactory than a 7-day total. In fact, the 5-day total for an average of $3\frac{3}{4}$ hr. sunshine daily was satisfactory in all months and saved nearly half the spraying rounds.

In order to give a visual illustration of blister-blight incidence throughout the greater part of the period concerned, the percentage shoot-infections of three treatments (A, B and E, of Table 2) are presented in Fig. 6. This figure also gives the daily sunshine, averaged over 5-day periods, recorded between 18 and 22 days (on an average,

20 days) before leaf examination, on the assumption that the sunshine prevailing then is critical for subsequent shoot infection (see p. 310). The symbols at the top of the figure show the dates on which spraying was carried out on the control plots (B) and on the plots sprayed when daily sunshine averaged less than $3\frac{3}{4}$ hr. (E).

Fig. 6 demonstrates that the degree of infection on the unsprayed plots was largely dependent on the amount of sunshine prevailing about 20 days earlier. The same trends occurred in the sprayed plots, but the level of infection was lower. The figure

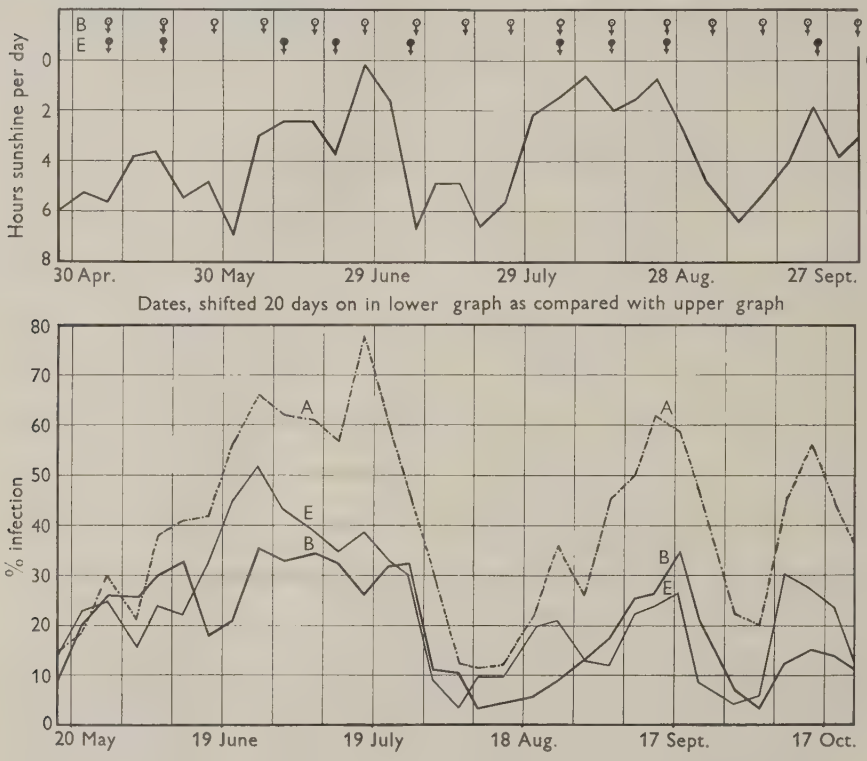

Fig. 6. Incidence of blister blight over 5 months on (A) unsprayed plots, (B) plots given the routine sprayings, and (E) plots sprayed only if the average daily sunshine was less than $3\frac{3}{4}$ hr. per 5-day period at 20 (18–22) days previously. The upper part of the figure shows the 5-day averages of daily sunshine with an inverted scale and with the dates shifted 20 days compared with the lower figure; open circles give the dates of routine spraying (B), solid circles the dates of spraying decided on the basis of previous sunshine (E). The lower part of the figure gives the incidence of blister blight with three different treatments.

also shows that the protection obtained when control measures were timed on the basis of sunshine records was nearly as good as that obtained with routine spraying; but when sunshine records were used for timing, only nine sprays were required instead of sixteen for the control.

The above observations indicate that sunshine—at least for practical purposes—is the dominant factor for blister-blight incidence.

We wish to thank Ir K. J. Wolthuis for his advice regarding his unpublished spore-germination technique for timing blister-blight infection and the Director of the Tea Research Institute of Ceylon, Dr D. L. Gunn, C.B.E., for editing this paper.

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Potato haulm resistance to *Phytophthora infestans*

II. Lesion production and sporulation

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SUMMARY

The slower increase of blight (*Phytophthora infestans*) (Mont.) de Bary in field plots of Majestic (MJ) and Arran Viking (AV) than of Up-to-Date (UD) and King Edward (KE) potatoes seems more closely related to the production of fewer spores than to greater resistance of leaves to infection.

Sporing was decreased, not by differences in generation time, but because fewer spores were produced per lesion on MJ and AV, especially when lesions were small (young). Infected cells of AV died more rapidly than cells of UD and KE and so left a smaller area of sporing tissue in young lesions. Although the fungus advanced at much the same rate in leaves of the four varieties, sporing annuli were narrower on older (larger) lesions on AV unless high relative humidity persisted continuously. Sporangiophores and sporangia were formed more rapidly on UD than AV when non-sporing lesions were transferred to high humidity. The ecoclimate of a crop of UD did not increase the sporulation on infected leaves of AV exposed within it, but sporulation was slightly decreased when infected UD leaves were exposed in a crop of AV. Generally KE behaved like UD, with MJ intermediate between these and AV.

The lower leaf-surface was more susceptible to infection than the upper. Differences in susceptibility of lamina between varieties were inconsistent. MJ and AV were more resistant than UD and KE when inoculated in leaf axils.

INTRODUCTION

The potato varieties Up-to-Date, King Edward, Majestic and Arran Viking showed small but significant differences in the time *Phytophthora infestans* took to destroy them (Lapwood, 1961*a*). The differences resulted mainly because blight developed more slowly during the early stages of epidemics in plots of Majestic and Arran Viking than of Up-to-Date and King Edward: defoliation later proceeded at the same rate on all four varieties, but there was some indication that leaves of King Edward were destroyed more rapidly after infection than those of Arran Viking.

Blight destroys potato foliage by the advance of the fungus from many separate and discrete infections and the rate of destruction of a variety depends on how quickly the foliage is infected, the number and position of the infections and the rate the fungus advances within the host. The aim of the investigation was to find differences between varieties differing in haulm resistance, which might explain their behaviour

in blight epidemics. For convenience of presentation, factors affecting the rate of infection of foliage are considered here and those affecting the rate of destruction of foliage after infection in a later paper.

Successful infection of the host, i.e. the entering and establishment of a pathogenic relationship (Anon. 1950), depends upon the amount of inoculum, host susceptibility and the environmental conditions for infection. Of these factors, the first two are considered in this paper.

A. FACTORS AFFECTING THE AMOUNT OF INOCULUM

Materials and methods

The main work was confined to the varieties Up-to-Date, King Edward, Majestic and Arran Viking, referred to collectively as the standard varieties, but to emphasize characteristics of resistant varieties some results with Ackersegen, Ås and Ontario will also be referred to. Field observations were made in the plots described earlier (Lapwood, 1961*a*) unless otherwise stated.

In laboratory experiments, leaves were cut either (*a*) from glasshouse-grown plants with 12–14 expanded leaves, usually leaves from positions 7, 8, 9 and 10, or (*b*) from field plants using the youngest fully expanded leaves. The terminal and distal pairs of leaflets of detached leaves were inoculated on the undersurface with sprays, or with droplets of sporangial suspension fixed in position with a small (7 mm.) filter-paper disc. The inoculated leaves were then incubated for 24 hr. at 15° C. in seed boxes (15 × 19 in.) lined with 'Polythene' sheet and dampened 'Airoporena' (a plastic foam sheet supplied by Progress Mercantile Co. Ltd, London, E.C. 1), so maintaining high humidity. Subsequently leaves were transferred to specimen tubes (4 × 1½ in.) and kept in a cool glasshouse.

Results

(1) *Incubation and generation time*

Incubation time is the interval between infection and the appearance of the first effects on the host, and with blight it is usually taken as the interval between inoculation and the first appearance of a necrotic spot. The time taken by the fungus to produce spores after inoculation, or the generation time, was found by Schaper (1951) and others to differ in individual varieties and these deviations were misleadingly referred to as differences in incubation resistance (Gallegly & Niederhauser, 1959).

Numerous experiments in the field and laboratory with the four standard varieties, and more recently with Ackersegen, Ås and Ontario, failed to show any difference in incubation time between the varieties, both under laboratory and field conditions; but Arran Viking, Ackersegen and Ås react more necrotically and the initial lesion shows as a darker brown spot than in the other varieties. In contrast, the generation time differed with variety and also with the method of inoculation. Detached leaves were either sprayed with a dilute sporangial suspension or inoculated with a droplet fixed with a filter-paper disc. When lesions were just visible, after using the two inoculation techniques described, discs were cut from leaflets with a No. 10 cork borer and incubated at 15° C. and at high humidity. Discs were then examined daily with a stereoscopic microscope for sporangiophores. Many lesions developed within the

7 mm. area covered by the inoculum and filter-paper disc, and spores were produced 24–48 hr. before the spray inoculations where lesions were well separated (Table 1). Spores were produced consistently later on Ackersegen and Ås but differences between the other varieties varied between experiments and were disregarded.

There were also indications that the numbers of sporangiophores produced differed with variety. Deshmukh & Howard (1956) found that sporangia were produced more

Table 1. *The relationship of variety and inoculation method to generation time*

(Figures indicate number of replicates sporing out of 24.)

	Days from inoculation				
	3	4	5	6	7
	(*a*) Droplet inoculation				
Up-to-Date	0	12	23	24	—
King Edward	0	8	22	24	—
Majestic	0	14	21	24	—
Arran Viking	0	1	13	23	24
Ackersegen	0	0	18	23	24
Ås	0	1	13	20	23
Ontario	0	3	18	22	24
	(*b*) Spray inoculation				
Up-to-Date	0	0	1	14	24
King Edward	0	0	3	21	24
Majestic	0	0	0	17	23
Arran Viking	0	0	0	14	22
Ackersegen	0	0	0	5	13
Ås	0	0	1	9	22
Ontario	0	0	0	17	23

Table 2. *Numbers of spores produced after similar periods of incubation from inoculation*

	3 days	4* days	5 days† (*a*)	5 days† (*b*)	Width of sporing annulus 5th day (mm.)
Up-to-Date	0	10·1	35·1	35·1	2·3
King Edward	few	11·8	60·5	49·9	4·8
Majestic	few	5·2	43·7	35·1	4·8
Arran Viking	0	0·9	13·9	19·6	0·9
Ackersegen	few	0·4	8·6	3·8	0·2
Ås	0	0·1	5·3	9·8	0·5

* Numbers per lesion $\times 10^3$.

† Numbers per lesion punching (Fig. 1) $\times 10^3$ (*a*) and (*b*) replicates—see text.

quickly and abundantly on Arran Pilot than on Ackersegen. To investigate this difference further five leaves were inoculated with droplets of suspension and incubated as usual; when symptoms first appeared, the leaves in tubes were transferred to a high humidity in a 'Perspex' chamber. A few sporangiophores were seen on the 3rd day after inoculation but their number was not estimated until the 4th day.

Spores were washed from the undersurface of leaves and counted on a haemacytometer slide. On the 5th day, because the sporing area was large, the method was changed and samples were taken with a modified leather punch (Hirst, unpublished); two rectangular clippings on a radius through the sporing area of each lesion were taken, one from each side of the central necrotic area. The two sets of clippings (*a*) and (*b*) were kept separate and spores counted as before. The extent of the band of sporulation (sporing annulus) was also measured in millimetres on the 5th day.

The number of sporangia depended on the variety (Table 2); usually more developed on Up-to-Date and King Edward than on the other varieties, but as the size of lesions

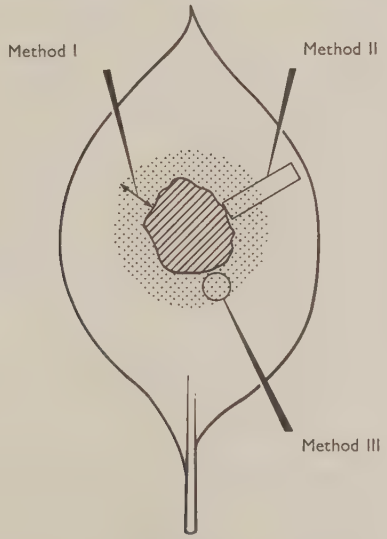

Fig. 1. Methods of assessing sporing capacity in the field. I. Extent of band (annulus) of sporulation measured in mm. II. Estimates of number from rectangular areas (0·5 × 2·5 cm.) punched through the lesion. III. Estimate of intensity of sporulation from samples punched from within the sporing area (disc diameter 0·5 cm.). Shaded area—necrosis: dotted area—sporulation.

increased differences from Majestic got smaller. The few spores produced by Arran Viking, Ackersegen and Ås were associated with a narrow sporing annulus. This is the first indication of a difference between the four standard varieties with Arran Viking more resistant, behaving like the recognized resistant varieties.

(2) *Sporing capacity in the field*

Several methods (Fig. 1) were used to study the sporing capacity of varieties in the field. Estimates were made daily throughout the epidemics 1956 to 1958. The area or width of the sporulation annulus was measured (method 1) for at least five lesions with about 1·0 cm. diameter necrotic area, at three heights in the canopy (upper, middle and lower). In 1956 a scoring method was used but in later years

measurements were made in millimetres. The average width calculated from the figures for each height was taken as the assessment for that day.

The sporing annulus is consistently narrowest in Arran Viking and widest in

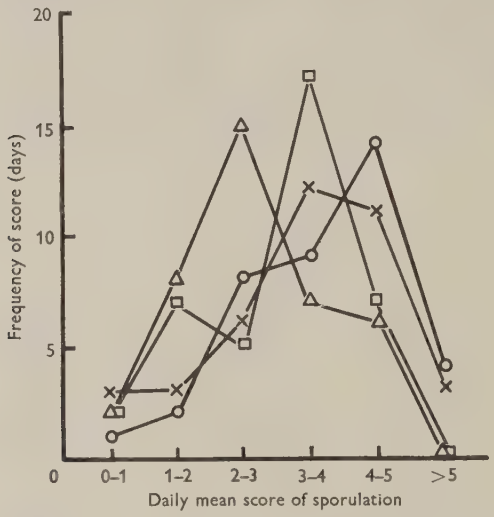

Fig. 2. Field estimates of sporulation 1956. Mean daily score estimated from the width of sporing annulus of lesions at three heights in the crop canopy. Means from 38 days summarized as a frequency distribution. Symbols: ○, Up-to-Date; ×, King Edward; □, Majestic; △, Arran Viking.

Table 3. *Estimate of sporing capacity from field observations*

(*a*) Mean width of sporing annulus from three heights in crop canopy of lesions with about 1 cm. necrosis.

	No. days	Up-to-Date	King Edward	Majestic	Arran Viking	L.S.D. ($P = 0.05$)
1956 (score)	22	3·8	3·5	3·0	2·7	0·24
1957 (mm.)	25	3·6	3·0	2·8	2·4	0·37
1958 (mm.)	20	4·5	4·2	3·7	3·5	0·36
1957 (counts)*	21	4·2	4·2	3·9	2·6	1·35

* Numbers per leaf punching $\times 10^3$—see Fig. 1 and text.

(*b*) Intensity of sporulation within the area sporing.

	No. days	Up-to-Date	King Edward	Majestic	Arran Viking	L.S.D. ($P = 0.05$)
		Number of spores per disc* $\times 10^3$				
1956	1	8·0	7·5	7·9	7·7	—
1957	6	9·3	8·7	7·1	7·0	1·86
1958	5	15·5	16·6	12·5	15·2	2·62
		Score method				
1958	16	3·5	3·4	3·1	3·2	0·30

* See Fig. 1.

Up-to-Date (Fig. 2, Table 3*a*). Of the two intermediate varieties Majestic was significantly less than King Edward in 1956 and 1958. In 1958, when the mean for Arran Viking was 3·5, Ås, Ackersegen and Ontario were 2·1, 1·7 and 1·6 mm., respectively. The numbers of spores produced were estimated in 1957 by taking clippings (method 2, Fig. 1) from five lesions at each of the three canopy heights, bulking and suspending in a known volume of liquid (Table 3*a*). The counts confirmed that Arran Viking produced fewer spores than the other varieties and showed that the two methods of assessment were closely correlated (Fig. 3). The density of sporulation within

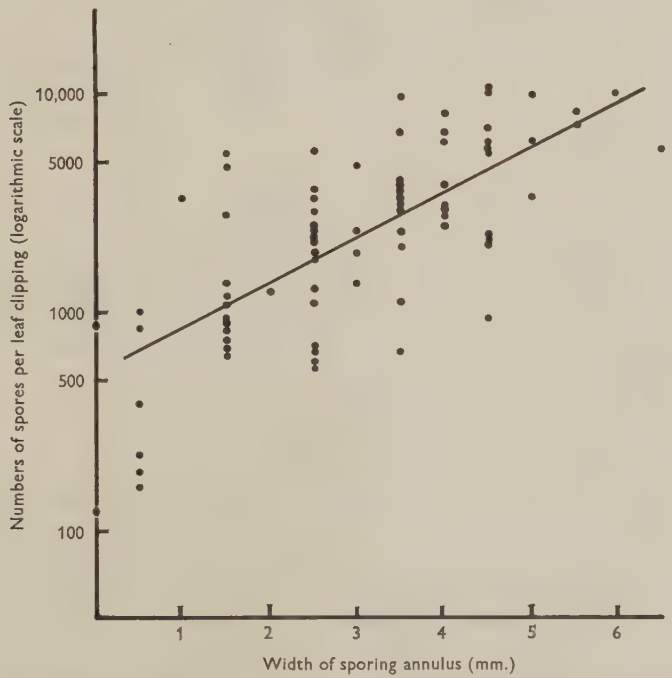

Fig. 3. Mean width (mm.) of the sporing annulus from lesions at three heights in the crop canopy compared for 20 days with estimates of spore number from leaf punchings (see Fig. 1), bulked for the four standard varieties 1957. Correlation coefficient $r = 0{\cdot}58$.

the sporing zone was estimated by punching out discs (method 3, Fig. 1), again using a total of fifteen lesions from different parts of the canopy, and suspending in liquid and counting as before. In 1958 a score 1 to 4 was used in conjunction with daily estimates; the key used was

1—sporulation patchy with no definite annulus
2—a definite sporing annulus but few sporangiophores
3—sporing area white but green of leaf still visible through it
4—sporing area so dense no leaf seen

Differences using the disc method, although sometimes significant (Table 3*b*), were not consistent because there were few days when the sporing area was wide enough in the four standard varieties for samples to be taken. The scoring method, which could be used daily, indicated that the sporulation of Up-to-Date was more intense than in Majestic and Arran Viking.

Table 4. *The extent of sporing annulus (mm.) of lesions of different ages in the field in* 1958

(Means of 11 consecutive days of three heights in the canopy.)

	Variety (±0·19)				
	Up-to-Date	King Edward	Majestic	Arran Viking	Mean (±0·09)
Old 15–20 mm.	3·9	3·7	3·2	2·9	3·45
Young 2–3 mm.	3·2	2·9	2·2	1·1	2·34
Mean (±0·13)	3·5	3·3	2·7	2·0	—

A significant interaction was found between varieties and the sporing annuli on lesions of different ages in the field. Lesions with 2–3 mm. necrosis and 15–20 mm. necrosis were compared for 11 consecutive days, using the usual methods of measurement. Young lesions had a smaller sporing area than older ones (Table 4) and those of Arran Viking were significantly smaller than those of the other standard varieties although differences were less when the lesions were larger.

(3) *Factors affecting sporing capacity*

Having established that Arran Viking produced fewer spores than the other standard varieties, the rate the fungus advanced in the leaf was assessed, to see whether this could account for the smaller sporing area. Leaf laminae were inoculated in their centre with droplets of suspension and incubated as already described. Advance of the fungus in millimetres was estimated from the mean of two measurements, taken at right angles, of the diameter of the lesion as indicated by the outer perimeter of the sporing area visible to the unaided eye. Table 5 shows that the fungus advances equally well on all four standard varieties, but that the necrotic area was greatest in Arran Viking. Comparing the diameter of necrosis on the 5th day with the sporing diameter of the previous day shows that only in Arran Viking were the areas that carried spores on the 4th day dead on the 5th day.

Expressing sporulation (Table 3) for the four standard varieties in the field as means for each of the three heights in the canopy (Table 6) shows that sporulation was more extensive within the canopy than outside, especially in the slow epidemic of 1957. In the rapid epidemic of the wet season 1958, the differences were smaller. To investigate further the part played by crop canopy, tests were made to see whether sporulation on one variety was affected by putting infected leaves in a crop of another variety. Artificial plants were built up by strapping small $2\frac{1}{2} \times \frac{5}{8}$ in. specimen tubes, in pairs, in three positions on canes 3 ft. high, so adjusted that the lowest pair when placed in the crop would be at ridge height and the uppermost just exposed from the canopy.

Field leaves with terminal and distal pairs of leaflets inoculated at the tip were, when lesions developed, placed in the tubes so that each pair of tubes had one Up-to-Date and one Arran Viking leaf. In this way both varieties were exposed at three similar heights in relation to the canopy. Ten artificial plants were constructed; five were exposed in a crop of Arran Viking and five in Up-to-Date. The widths of the sporing annuli, measured in millimetres daily for 5 days (Table 7), depended largely on the variety of the infected leaf exposed, but the Arran Viking crop tended to decrease the sporulation of Up-to-Date.

Table 5. *Estimating advance of the fungus from sporulation of lesions and the relationship between sporing and necrotic diameters*

(Means of 12 replicates)

	4 days	5 days		
	Mean diam. sporulation (mm.)	Mean diam. sporulation (S) (mm.)	Mean diam. necrosis (N) (mm.)	Ratio N/S
Up-to-Date	8·7	13·2	6·1	0·48
King Edward	6·9	13·0	6·3	0·48
Majestic	7·7	14·5	6·4	0·45
Arran Viking	9·9	14·9	11·5	0·77
L.S.D. $P = 0·05$	1·65	1·95	1·50	0·11

Table 6. *Width of sporing annulus (mm.) at three different heights in the canopy*

(Means of 26 days in 1957 and 12 days in 1958.)

		1957	1958			1957	1958
Up-to-Date	Upper	2·7	4·6	Majestic	Upper	1·9	3·2
	Middle	3·9	5·0		Middle	3·1	4·4
	Lower	4·4	5·5		Lower	3·5	4·7
King Edward	Upper	1·9	4·0	Arran Viking	Upper	1·6	3·5
	Middle	3·4	4·9		Middle	2·6	4·2
	Lower	3·8	5·2		Lower	3·0	4·5

The differences between sporulation on Up-to-Date and Arran Viking are pronounced when varietal effects are considered in conjunction with age of lesion and position within the crop (Table 7*c*). Thus on 28 August, sporulation near the ridge was similar on both varieties but differences were obvious between the varieties higher in the canopy, whereas on 27 August, when the lesions were a day younger, the sporing area of Arran Viking was considerably less than Up-to-Date and was unaffected by the crop variety or position in the canopy. When assessed, the outer perimeter of the sporing zone of lesions was marked by pin pricks, and this showed that near the ridge sporulation of lesions was often continuous between consecutive days, whereas higher in the crop canopy breaks in sporulation were shown by non-sporing bands. Where sporulation was continuous, measurements from the pin pricks indicated that lesions on Arran Viking had a sporing band as wide as on Up-to-Date.

P. infestans produces aerial mycelium only when relative humidity is above 85%, and abundant sporangia only in saturated air (Crosier, 1934). The relative humidity within crops usually falls during the day but reaches saturation during the night, and the fall in humidity interrupts spore production and leads to distinct bands or annuli of sporing tissue. Thus in the laboratory, sporulation is continuous when leaves are kept continuously at high humidity, but it is discontinuous when humidity alternates for a period between high and low.

Table 7. *Effect of canopy on the width of sporing annulus (mm.) of lesions on leaves of Up-to-Date and Arran Viking when placed in the two crops*

(*a*) Interaction of leaf and position in canopy.

Infected leaves	Leaf position in canopy (±0·13)			Mean (±0·075)
	Upper	Middle	Lower	
Up-to-Date	3·2	3·4	4·0	3·7
Arran Viking	1·2	1·6	2·9	1·9
Mean (±0·09)	2·2	2·7	3·5	

(*b*) Interaction of leaf and crop.

Crop	Infected leaves (±0·11)		Mean (±0·07)
	Up-to-Date	Arran Viking	
Up-to-Date	3·8	1·9	2·9
Arran Viking	3·5	1·8	2·7
Mean (±0·07)	3·7	1·9	

(*c*) Effect of crop canopy and age of lesion.

Infected leaves	Crop	Canopy position					
		27 Aug.			28 Aug.		
		Upper	Middle	Lower	Upper	Middle	Lower
Up-to-Date	UD	2·6	2·7	3·4	4·5	4·6	4·1
	AV	2·7	3·0	3·9	4·3	4·4	4·1
Arran Viking	UD	0·6	0·6	0·7	2·4	2·5	4·0
	AV	0·7	0·5	0·6	2·6	2·5	4·0

Experiments were done with different varieties to find how soon leaves from low humidity started to spore when left at high humidity. Detached leaves, from field or glasshouse plants, were inoculated as usual and when a substantial lesion (8–10 mm. necrosis) had developed at low humidity, either discs cut through the lesion with a No. 10 cork borer or whole detached leaflets were incubated at high humidity in boxes at 15° C. The leaflets were divided into two batches, one of which was sampled after 5 hr. and the other after 10 hr.; all were reassessed after 24 hr. Each lesion was examined on the undersurface of the leaf with a microscope for signs of aerial hyphae and the formation of spores, both criteria being estimated using a scoring method. Aerial mycelium was well developed and a few spores were produced on Up-to-Date within 5 hr., whereas Arran Viking had only sparse aerial hyphae (Table 8). After 10 hr. Up-to-Date was sporing extensively, whereas Arran Viking had only few spores.

In other tests King Edward behaved like Up-to-Date; Majestic and Ås were intermediate; and Ackersegen and Ontario behaved more like Arran Viking.

Table 8. *Time taken by the fungus to produce spores at* 15° C *when infected leaves of Up-to-Date (UD) and Arran Viking (AV) were transferred to high humidity*

(Figures indicate numbers of leaf discs having scores 0–4 out of 52 (UD) and 54 (AV) replicates.)

		Aerial mycelium					Sporulation				
	Score* ...	0	1	2	3	4	0	1	2	3	4
5 hr.	UD	5	0	3	17	27	49	3	0	0	0
	AV	46	3	1	4	0	54	0	0	0	0
10 hr.	UD	1	0	1	13	37	1	3	21	18	9
	AV	21	11	18	4	0	36	9	9	0	0
24 hr.	UD	—	—	—	—	—	1	1	3	18	81
	AV	—	—	—	—	—	12	4	32	48	12

* 0 = no response. 4 = maximum response.

B. SUSCEPTIBILITY TO INFECTION

(1) *Susceptibility of leaf laminae to infection*

The probability of a leaf becoming infected depends upon a source of inoculum, the persistence of water and the susceptibility of the leaf. Susceptibility to infection, as meant here, is expressed as the number of necrotic lesions that develop per unit leaf area (per cm.^{2}) when leaves are sprayed with a standard inoculum.

To assess the variability of material, three leaves were cut (consecutive leaf positions) from three plants of each of the varieties: leaves from one plant of a variety were kept together in an experiment as a block. The leaves, laid flat on the bench with the terminal and distal pair of leaflets well separated, were sprayed with a dilute suspension of sporangia (5000–7000 per ml.) from an atomizer, which was passed along the line of leaves. Inoculated leaves were transferred to 'Polythene-Airoporena'-lined seed boxes and incubated for 24 hr. at 15° C., removed, and then placed in water in specimen tubes until small lesions appeared. Lesions were counted on two consecutive days; lesions counted on the first day were pierced through with a sharp pencil. The area of leaflets was assessed using a grid designed for apple leaves (Freeman & Bolas, 1955) traced on to a sheet of 'Perspex'. Estimates in cm.^{2} were similar to areas given by a planimeter (correlation coefficient $r = +0{\cdot}98$ between methods).

In the first experiments the lower leaf-surface was inoculated and the results for the seven varieties were analysed for sources of variation (Table 9). The analysis shows significant differences between leaflets (considered an assessment of method), leaf position and between plants. As a result, methods were revised, two consecutive leaves from positions 7 to 10 were taken from four plants per variety. Leaves on the bench were wetted by lightly spraying with water before they were inoculated with a fine spray from a two-bulb atomizer. When the lower surfaces of leaves were inoculated, all varieties produced similar numbers of lesions (Table 10*a*), but when the upper surface was inoculated differences between varieties were sometimes large. Early in the season, Up-to-Date gave more lesions than Arran Viking, but later (Table 10*b*) gave fewer.

The lower surfaces were more susceptible to infection than the upper (Table 11); differences were more obvious when many replicates were used, e.g. with Up-to-Date when the mean number of lesions on the upper surface was 9·9 the lower surface was 14·6 (L.S.D. ($P = 0{\cdot}05$) 2·5). However, it seems that differences in susceptibility of lamina between varieties are small, less than those between different leaves and leaf surfaces of one variety.

Table 9. *Analyses of variances to show sources of variation in estimates of susceptibility to infection (expressed as number of lesions per unit leaflet area) of the lower surface of leaves of seven varieties*

	D.F.	Mean square (variance)	Variance ratio
Between varieties	6	59·66	5·75*
Between plants within a variety	14	29·15	2·81*
Leaf position	2	32·80	3·16†
Residual between leaves	40	10·37	—
Between leaflets	2	103·00	13·4*
Residual between leaflets	124	7·69	—

* Variance ratios significant at more than $P = 0{\cdot}01$.
† Variance ratios significant at $P = 0{\cdot}05$.

Table 10. *Susceptibility of leaf lamina to infection, expressed as numbers of lesions per cm.²*

	Up-to-Date	King Edward	Majestic	Arran Viking	Acker-segen	Ås	Ontario	L.S.D. $P = 0{\cdot}05$
				(*a*) Lower surface				
Expt.								
1	8·4	7·6	7·6	6·4	4·8	6·0	4·5	N.S.
2	5·3	5·1	7·6	6·5	7·5	4·3	6·9	N.S.
3	6·6	5·6	8·5	5·1	7·8	5·9	6·0	2·03
Mean	6·8	6·1	7·9	6·0	6·7	5·4	5·8	N.S.
				(*b*) Upper surface				
Expt. (1959)								
13 May	5·4	3·0	2·4	2·1	—	—	—	1·06
19 May	6·7	4·3	5·3	3·6	—	—	—	2·09
12 Aug.	3·8	3·8	2·1	4·7	1·6	1·3	—	2·39
22 Aug.	2·1	0·5	1·1	1·1	0·6	0·6	—	0·51
7 Sept.	3·9	2·5	3·3	5·1	3·6	3·5	3·6	N.S.
10 Sept.	5·1	3·3	3·7	7·1	3·3	3·1	2·5	1·93
Mean	3·7	2·5	2·5	4·5	2·3	2·1	—	1·21

Table 11. *Comparing susceptibility of leaf surface to infection (lesions per cm.²)*

	Variety ±0·53				
	Up-to-Date	King Edward	Majestic	Arran Viking	Mean ±0·27
Upper	2·8	2·0	4·9	3·2	3·2
Lower	3·9	2·8	7·0	5·4	4·8

(2) *Susceptibility of leaf axil*

In the field, first infections with blight are sometimes associated with water droplets persisting in leaf axils (van der Zaag, 1956). This observation prompted an investigation into the effect of inoculating varieties in the leaf axil. Potted plants at the 14-leaf stage were inoculated at ten or more leaf axils with a droplet of active zoospore suspension. After 24 hr. of high humidity in a large 'Polythene' chamber the plants were

Table 12. *Varietal susceptibility to inoculation of the leaf axil (bulked data of 1957 experiments)*

	No. of inoculations	Buds		Lesions	
		Infected	Dead	Leaf petiole	Stem
Up-to-Date	99	99	94	71	64
King Edward	122	116	109	77	74
Majestic	171	134	94	33	49
Arran Viking	102	63	25	11	8

Table 13. *Percentage stem and leaf petiole lesions resulting from the inoculation of the leaf axils*

	Up-to-Date	King Edward	Majestic	Arran Viking	Ontario	Acker-segen	Ås	L.S.D. $P = 0.05$
Stem	32·3	24·7	8·7	4·5	3·2	6·2	0	—
	(1·60)*	(1·43)	(1·02)	(0·46)	(0·38)	(0·92)	—	(0·43)
Leaf petiole	43·0	39·5	12·5	1·3	14·8	5·8	1·5	—
	(1·70)*	(1·62)	(0·98)	(0·19)	(1·16)	(0·50)	(0·20)	(0·56)

* A logarithmic transformation used for statistical analysis.

removed to a shaded glasshouse bench. The number of axillary buds infected and destroyed were counted and the development of lesions on the stem and petiole was assessed 7 and 14 days after inoculation. Consistent differences between varieties were obtained: King Edward and Up-to-Date were always more affected than Majestic, and Arran Viking less (Table 12). Not only were fewest buds infected and destroyed in Arran Viking but the lesions usually remained very small, whereas in King Edward and Up-to-Date they often girdled the leaf petiole, the leaf collapsed and after a while the stem was also girdled.

In 1959, Ontario behaved like Majestic and Ackersegen whereas Ås behaved like Arran Viking (Table 13).

The varietal effects on the development of stem lesions agreed with field observation of lesions on marked stems (Lapwood, 1961*a*) made during the 1956 and 1959 blight epidemics (Table 14). In both seasons fewer stem lesions developed on Arran Viking than on the other standard varieties, and Ackersegen and Ås developed even fewer. The effects of stem lesions were particularly noticeable in 1959 when dry weather at an early stage in the blight attack prevented further spread. The crops were mainly damaged by the fungus advancing from infected leaflets into leaf petioles and stems.

In these conditions the differing extents of stem resistance were particularly important; many lesions rapidly girdled the stems of Up-to-Date and King Edward, but few did so in Arran Viking and Ås, and those that eventually did were near the top of plants.

Table 14. *Numbers of lesions (mean per stem) occurring on marked stems in field experiments*

		Up-to-Date	King Edward	Majestic	Arran Viking	Ås	Ackersegen
1956	23 Aug.	8·6	4·6	2·1	0·5	—	—
1959	8 Sept.	2·3	3·9	2·3	1·7	0·4	0·1
	15 Sept.*	0·3	1·8	0·4	0	0	0

* Lesions girdling the stem.

DISCUSSION

Laboratory experiments failed to detect consistent differences between the susceptibility to infection of leaves of the varieties Up-to-Date, King Edward, Majestic and Arran Viking that could account for the differences observed in field crops. However, leaf infection in the field probably depends not only on the host's inherent susceptibility, but also on factors controlling the persistence of water droplets, e.g. the nature of the leaf surface, the growth habit of the plant and the type of crop-canopy produced.

Umaerus (1960) showed that the minimum infection period varied between varieties, for the susceptible Anna it was 3 hr. and for the resistant Ackersegen 8 hr. Preliminary work with my four standard varieties showed no such differences and at 15° C. the infection period was 4–5 hr.

When water droplets persist for long periods many sporangia germinate, either directly or indirectly, and they, or their encysted zoospores, produce long germ-tubes, many of which fail to penetrate the host. There is probably a delicate balance between the infection processes and the drying of the water droplet or film, and this may influence the number of successful penetrations, quite apart from the resistance of the host. Optimum temperatures also vary for the various processes (Crosier, 1934); low temperatures stimulate the release of zoospores, and a rising temperature decreases the swimming time, hastening encystment and subsequent germination. The rate at which the water film dries may also be important in bringing the germinating spores close to the leaf surface, as contact stimuli may be necessary for appressoria formation (as found in powdery mildews, Dickinson, 1949) and subsequent penetration; there may also be an optimum germ-tube length for this, as the food reserves of the encysted zoospore must be limited. Thus condensing susceptibility to infection to a simple standard test is obviously of limited value.

The fungus spored in different amounts on the four standard varieties, decreasing in the order Up-to-Date, King Edward, Majestic and Arran Viking.

The generation time of the fungus was similar on the four varieties, as was the rate the fungus advanced in leaf tissues; nevertheless, different varieties produced different numbers of spores per lesion. In laboratory conditions differences between the varieties were greatest when lesions were small, and decreased as lesions enlarged. In the field, however, lesions of about 1 cm. necrosis spored in different amounts

depending on their position in the crop canopy. On all varieties the band of sporulation was generally widest near the ridge, and here differences between varieties were least, whereas higher in the canopy of each variety the sporing band was narrower, and the differences between varieties greatest (see Table 6).

These differences within a crop may be explained by differences of ecoclimate at different crop heights (Broadbent, 1950; Hirst, Long & Penman, 1954); high humidity favouring sporulation might be expected to be maintained longest in the lower canopy. Another factor may be involved, for Lowings & Acha (1959), using glasshouse-grown Majestic, showed that the rate the fungus advanced depended on the age both of the leaf and of the plant, and therefore, in addition to any effects of temperature differences in the crop, the fungus may be advancing at a different rate in lesions at the different heights selected for measurement.

Although differences in sporulation within a crop may be caused by ecoclimate, differences between crops of different varieties do not seem to be entirely attributable to different ecoclimates, but more to differences in the host–parasite relationship. When non-sporing lesions on leaves are transferred from low to high humidity, spores are formed more rapidly on Up-to-Date than on Arran Viking. Recently such a difference was demonstrated between susceptible and resistant clones of *Solanum andigenum* (Guzmán, Thurston & Heidrick, 1960). Prolonged high humidity decreases this effect, and when sporulation continues from day to day it can be equally extensive in both varieties because the daily increment in fungal growth is similar. However, in fluctuating humidity, this delay appears to decrease spore production; consequently the sporing band on Arran Viking remained narrow when infected leaves were exposed in the upper canopy either of a crop of Up-to-Date or Arran Viking. The differences between varieties may be accentuated by the more rapid death of Arran Viking tissues, as necrosis may extend into the area invaded by the fungus during the period of low humidity and decrease further the area able to support sporulation.

The resistance shown by Ås and Ackersegen differs from that of Arran Viking as there is evidence of a longer generation time, and the narrow annulus of sporulation may be attributed to a slower rate of advance of the fungus in leaves (Lapwood, 1961*b*).

The evidence presented in this paper suggests that the slower rate of infection of foliage of Majestic and Arran Viking than of Up-to-Date and King Edward during the early phases of the blight attack (Lapwood, 1961*a*) may be attributed to their producing fewer spores rather than to greater resistance to primary lamina infection, although they are both more resistant than King Edward and Up-to-Date when inoculated in the leaf axils.

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Effect of nitrogen supply on the response of Majestic potato to gibberellic acid

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SUMMARY

Potato plants sprayed with gibberellic acid (GA) were given extra nitrogen either through the soil (as ammonium nitrate) or through the leaves (as urea). GA increased the initial production of dry matter but the effect did not persist, even with additional N. The number of leaves on lateral branches was decreased by GA partly because leaf production on laterals at lower nodes was inhibited. N increased the rate leaves were produced on lateral branches; the soil application doubled the leaf number and the urea increased it by 50%. GA, but not N, increased the area of a main-stem or lateral-stem leaf; however, nitrogen produced the largest total leaf area because it produced more leaves. There was no evidence for any interaction between GA and N either in the effect on leaf number or total leaf area. The fall in total N content per unit area caused by GA found in an earlier experiment was confirmed and additional N did not arrest this decline. Loss of efficiency of GA-sprayed plants is unlikely to result from indirect deficiency caused by rapid leaf expansion.

INTRODUCTION

Spraying potato plants with GA increased their leaf area and production of dry matter, but the increased dry matter was not maintained through to maturity (Humphries, 1958; Humphries & French, 1960). As the plants had absorbed all the N with which they were supplied before they matured, it seemed possible that their ability to respond to GA depends on a continuing supply of N. The experiments reported here were made to see whether there is any interaction between GA and N, which was supplied to some plants through the soil and to others through the leaves, to avoid the possibility that N uptake was limited by the activity of the roots.

MATERIALS AND METHODS

Table 1 shows the sequence of events during the experiment. Plants were raised from sets trimmed to a weight of approximately 5 g. and bearing a single eye, sprouted in sand and then planted singly on 27 April in glazed pots containing 10 kg. of a fertile light soil to which had been added 1·9 g. K, 0·7 g. P as potassium phosphate and 1·75 g. N as ammonium nitrate. GA was applied at the same rates as in the previous experiment, viz. two sprayings of 50 p.p.m. in water separated by an interval of 1 week (treatment A), of six sprays at weekly intervals (treatment B). The first spraying

was on 15 May, a week after leaf areas were first estimated. There were 105 plants altogether, arranged in five blocks in trucks in the glasshouse so that one plant of each treatment for each harvest was represented in each block. The trucks were pushed into the open air each day except in windy or wet weather. Up to the time of the first harvest no extra N was given to the plants, so that the comparison then was between controls and plants receiving two sprayings (treatment A) and three sprayings (treatment B) of GA. After the first harvest on 8 June, plants of treatment S received a single dose of extra N applied to the soil at the rate of 1·5 g. N per pot; a 0·5% solution of urea was sprayed on the leaves of plants of treatment L and spraying repeated twice weekly for a total of eleven occasions.

Table 1. *Sequence of events*

		27 April: sprouted sets planted in pots			
Treatments		Gibberellic acid spraying		Nitrogen application	
		A (2 sprays)	B (6 sprays)	L (urea to leaves)	S (ammonium nitrate to soil)
	8 May*	—	—	—	—
	15 May	×	×	—	—
	22 May*	×	×	—	—
	29 May	—	×	—	—
Harvest I	5 June*	—	×	—	—
	12 June	—	×	8,‡ 11 June	8 June‡
Harvest II	19 June*	—	×	15, 18 June	—
	26 June	—	—	22, 25 June	—
	3 July*	—	—	30 June, 2 July	—
	10 July	—	—	6, 9 July	—
Harvest III	17 July*	—	—	13 July†	—

* Leaf areas estimated.

† 2 days later plants were washed with distilled water to remove urea not absorbed by the leaves and stems.

‡ Additional potassium and phosphate applied to the soil on this date.

Before the sprayed plants were harvested they were sprayed with water to remove any urea remaining on the leaves and stems. All pots were given additional potassium and phosphorus to the soil on 8 June. Areas of all main-stem leaves and the leaves on alternate laterals were estimated at fortnightly intervals by rating them in comparison with a graded series of standards; analysis of previous results showed this estimates total leaf area adequately. Not all plants were always rated. At the start of the experiment, plants destined for harvest I were rated, and plants for harvest II were first rated at time of harvest I, on 5 June. Similarly, plants of harvest III were first rated at harvest II on 19 June. This procedure permitted estimation of the initial dry weight of plants subsequently harvested by means of a regression on leaf area.

RESULTS

Production and survival of leaves

In the present experiment plants were slightly younger when first sprayed with GA than in previous experiments (Humphries & French, 1960). Thus there were 14·7 leaves present on the main axis in the previous experiment when first sprayed and 12·8 (interpolated from observations made on 8 and 22 May) in the present experiment. This slight difference in age influenced the effect of GA on lateral branches. Thus in the present experiment GA inhibited lateral buds at nodes 3–7, whereas previously the inhibition was at nodes 10 and 11. This inhibition was attributed to competition for nutrients between developing laterals and main-stem leaves, but in the light of the present results this seems less probable, although the pattern of plant development

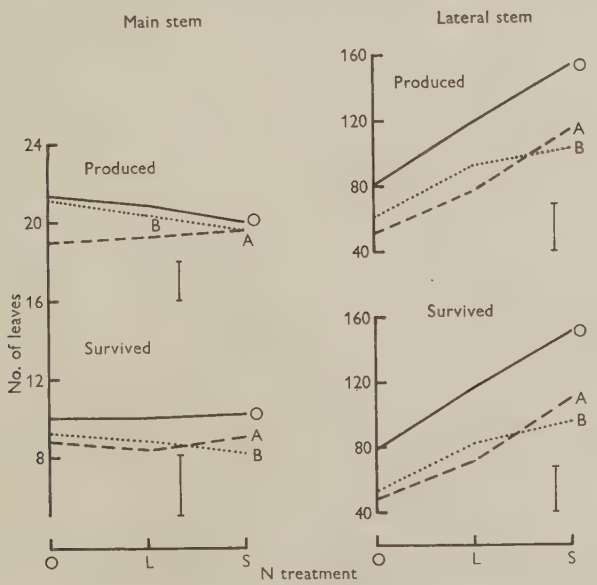

Fig. 1. Number of leaves produced and survived on main stem and lateral branches on 17 July. ——, control; - - - -, treatment A; · · · ·, treatment B. (Vertical lines indicate least significant differences.)

was rather different in the two experiments. There was much less leaf area at the lower nodes on sprayed plants than on the controls, probably because of a direct action of GA on the buds when they were at a sensitive stage. After this stage GA apparently no longer affected them. Also, the effect did not recur with repeated applications of GA for had it done so all lateral buds should have been affected. That this does not occur has been shown previously and is borne out in the present experiment, because leaves on higher lateral branches of treated plants tended to be larger than on the controls; the buds on these branches presumably passed through the same stages as the lower

ones during the period when GA was applied. No explanation can be suggested for inhibition occurring only at the lower nodes.

As found previously, spraying with GA did not affect the number of leaves produced on the main axis but it accelerated their senescence (Fig. 1). N had no effect on rate of production or death. In contrast, the number of leaves on the lateral branches was decreased by GA, partly because leaf production on the lower nodes was inhibited. N increased the rate at which leaves were produced, even on some of the branches

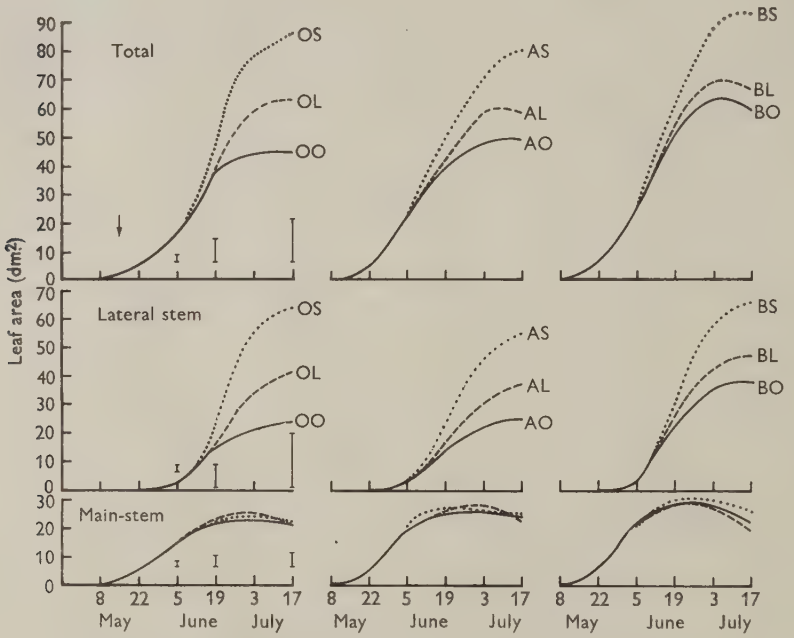

Fig. 2. Changes in total leaf area of main stem and lateral branches and total leaf area per plant with time. Arrow indicates date of application of first spray. ——, No nitrogen; - - - -, urea applied to the leaves; · · · ·, ammonium nitrate applied to the soil. (Vertical lines indicate least significant differences.)

where growth was inhibited by GA. N applied to the soil nearly doubled the number of leaves produced on lateral branches and urea sprays increased leaf number by about half. There is no evidence of any interaction between GA and N (Fig. 1).

Leaf area

Fig. 2 shows how GA and the superimposed N treatments affected growth of main-stem and lateral-stem leaves and total leaf area. Main-stem leaves reached their maximum area about 19 June and the total area of lateral leaves was probably not quite at its maximum when the experiment ended on 17 July. The lateral leaves then accounted for about two-thirds of the total leaf area. GA affected the size of main-

axis leaves, and six applications increased the area more than did two applications: however, N did not increase leaf size on the main stem and, when combined with six sprays of GA, decreased it.

N had a large effect on total leaf area of lateral branches; the soil treatment had a larger effect than the leaf treatment and increased the growth of lateral leaves alone above the total area of controls. N, however, increased lateral-leaf area by approximately the same amount with or without GA. Late spraying with GA had more

Table 2. *Mean area per leaf* (*dm.*^{2}). *Effects of treatment at* 17 *July*

	Main stem	Laterals	Lateral 5	Lateral 19
		Gibberellic acid		
O	2·13	0·22	0·18	0·30
A	2·71	0·32	0·22	0·35
B	2·58	0·41	0·29	0·48
		Nitrogen		
O	2·41	0·31	0·28	0·40
L	2·30	0·33	(0·07)	0·36
S	2·47	0·31	0·34	0·38

Table 3. *Dry matter* (*g.*)

		Main stem	Main leaves	Lateral stems	Lateral leaves	Tubers	Roots
Harvest I							
	O	2·8	6·8	0·6	0·9	1·4	2·2
	A	2·8	7·5	0·4	0·7	1·3	1·9
	B	3·5	8·3	0·3	0·8	1·1	1·8
	L.S.D.	0·53	1·00	0·20	0·47	0·15	0·19
Harvest II							
Gibberellic acid: treatment means	O	5·8	9·4	4·6	8·2	13·6	3·2
	A	5·4	10·5	3·3	7·2	17·7	3·0
	B	7·2	12·2	5·3	10·0	11·9	3·1
Nitrogen: treatment means	O	5·9	9·8	3·9	7·7	16·0	3·2
	L	6·4	11·0	4·1	7·8	15·6	2·9
	S	6·1	11·3	5·2	9·9	11·7	3·2
	L.S.D.	0·74	1·16	0·74	1·79	2·35	0·51
Harvest III							
Gibberellic acid: treatment means	O	9·4	10·9	15·5	17·5	88·7	3·5
	A	8·1	11·2	11·2	16·5	95·1	3·1
	B	9·6	12·1	12·3	21·3	82·9	3·0
Nitrogen: treatment means	O	9·3	11·6	8·4	13·6	84·3	3·0
	L	8·8	11·3	12·2	17·2	93·9	2·6
	S	9·1	11·4	18·4	24·4	88·5	3·9
	L.S.D.	1·06	1·27	1·94	1·62	8·55	0·89

Total dry matter

	Harvest I	Harvest II			Harvest III		
		O	L	S	O	L	S
O	14·6	45·3	45·6	43·5	131·1	147·8	157·7
A	14·5	46·4	47·0	48·2	136·0	152·6	146·8
B	15·8	48·4	50·6	50·3	123·6	137·5	162·3
	L.S.D. 2·34		L.S.D. 4·94			L.S.D. 16·36	

effect on lateral-leaf area than spraying discontinued earlier; the areas of plants in treatment A were not significantly different from the controls.

The mean area per leaf on 17 July (Table 2) was increased by GA; lateral leaves on GA-treated plants were nearly double those of controls but the effect on main-stem leaves was less. By contrast, N did not affect leaf size of main- or lateral-stem leaves. The figures quoted in Table 2 for a leaf on an early developed lateral (no. 5) and a late one (no. 19) support the conclusion that GA but not N increased leaf size and that the greater leaf area of the N plants resulted entirely from an increase in leaf number. The value of 0·07 against lateral 5 in treatment L (Table 2) was low because the growth of this lateral was inhibited by GA with treatment L but not with the controls or treatment S.

Dry-matter yield at successive harvests

Table 3 gives the total dry-matter content of the whole plant and component parts with the mean effects of GA and N at each of the three harvests. At the first harvest GA increased dry-matter content of main-stem leaves and main stem but decreased that of the lateral leaves and stems. Tuber weight was also decreased. Total dry matter per plant was not significantly increased. Thus, in the early stage of development, GA redistributed dry matter as noted in previous experiments. At the second harvest total dry matter was increased by six sprays of GA and all component parts contributed except the tubers. At this harvest, however, two sprays of GA increased tuber weight, showing as before that continued application of GA adversely affects dry-matter accumulation in tubers. GA appears to be translocated from the leaves to the tubers, stimulating their growth and proliferation, but when it is no longer applied to the leaves (as in treatment A) the tubers begin to accumulate dry matter. Although additional N was applied only 11 days before harvest II (Table 1), it increased dry matter in main-stem and lateral-stem leaves and lateral stems but did not affect tuber weight. Usually N applied to the leaves had less effect than the soil application. GA had no effect on total dry matter at the third harvest and, as in the previous experiment, the increase in dry matter achieved by GA-sprayed plants was subsequently lost, but the tubers of plants sprayed twice with GA and urea had more dry weight when the N was applied to the soil. As at the second harvest, N had no effect on dry weight of main stem at the third harvest, probably because the additional N was applied too late to influence development. N did not increase dry weight of main-stem leaves at the third harvest but had a large effect on lateral stems and leaves. Tuber dry weight was not influenced. Where the N treatments were effective, the leaf sprays increased dry matter less than the soil application and, although the amount of urea applied to the plants was not measured, there can be no doubt that less N was applied by this means than through the soil.

Nitrogen content

The total N contents of leaves, stems and tubers (Table 4) were estimated at each harvest. N applied to the leaves or to the soil increased N per plant; the effect was greater in lateral- than in main-stem leaves. There was, however, some effect on main-stem leaves, which when sprayed with GA and supplied with extra N to the soil had more total N than unsprayed plants. N supplied to the leaves did not have this

effect. The greater N content reflects the greater leaf weight (and area) produced by GA. Extra N had little effect on N content per unit area of leaf at the second and third harvests (Table 4, second part) except for leaves from the urea-sprayed treatment which had a consistently higher N content. Perhaps surface N was not removed before sampling, although this is unlikely. As in the previous experiment, N per unit area of lateral leaves was decreased by GA and the extra supply of N to the soil did not

Table 4. *Nitrogen per plant* (*leaves*) (*mg.*)

	Harvest I		Harvest II		Harvest III	
	Main leaves	Lateral leaves	Main leaves	Lateral leaves	Main leaves	Lateral leaves
O	421	70	431	482	252	396
OL	—	—	516	474	363	718
OS	—	—	470	720	314	1083
AO	432	54	429	396	282	368
AL	—	—	520	430	374	660
AS	—	—	600	634	316	960
BO	451	53	450	475	259	383
BL	—	—	535	490	342	656
BS	—	—	608	731	348	895
	Nitrogen: mg. per dm.^{2} of leaf					
O	26	36	20	28	12	17
OL	—	—	23	30	17	17
OS	—	—	22	30	14	17
AO	21	35	18	27	12	15
AL	—	—	20	27	17	18
AS	—	—	20	27	13	18
BO	19	30	16	18	12	11
BL	—	—	18	18	19	20
BS	—	—	20	22	13	13

Nitrogen per plant (mg.)

	Harvest II				Harvest III			
	O	A	B	Mean	O	A	B	Mean
O	1447	1364	1439	1417	1674	1719	1737	1709
L	1531	1527	1612	1558	2415	2499	2436	2451
S	1851	1958	2017	1942	2916	2770	2797	2827
Mean	1610	1616	1689		2335	2329	2323	

increase the N content. The additional N given via the leaves raised the N content per unit area, but did not increase dry-matter production. In this, as in the earlier experiment, GA decreased N content per unit area considerably, but supplying extra N did not stop the decrease. If the decrease in N content was a deficiency induced by rapid expansion, supply of additional N would be expected to restore the N content of the leaf to a value similar to the controls. It seems likely that loss of efficiency of GA-sprayed leaves has a more complicated origin than merely a decline in N content. The figures for total N per unit area suggest that one effect of GA is to disturb the normal N metabolism and this possibility requires investigation. The total N per plant at harvests II and III (Table 4) shows how little GA affects N uptake; indeed at harvest III there was not more than 12 mg. difference between the three treatments.

Net assimilation rate

The mean net assimilation rate (E) during the intervals between harvests (Table 5) for plants supplied with urea via the leaves was less than for plants given extra soil N or of the controls during the first period; possibly because the urea solution slightly scorched the leaves. This does not explain the reverse result obtained in the second period, when plants given extra soil N had a significantly lower E than those sprayed with urea or the controls. GA decreased E, as in previous experiments, and the gain in dry matter from GA resulted entirely from increase in leaf area.

Table 5. *Net assimilation rate* (*g./dm.²/week*)

5–19 June		Nitrogen O	L	S	Mean
Gibberellic acid	O	0·60[a]	0·52	0·63	0·58[b]
	A	0·57	0·47	0·54	0·53
	B	0·44	0·43	0·48	0·45
	Mean	0·54[b]	0·47	0·55	

L.S.D.: [a] 0·073, [b] 0·046.

19 June–17 July		Nitrogen O	L	S	Mean
Gibberellic acid	O	0·56[a]	0·51	0·44	0·50[b]
	A	0·52	0·53	0·42	0·49
	B	0·38	0·41	0·42	0·40
	Mean	0·49[b]	0·48	0·43	

L.S.D.: [a] 0·062, [b] 0·036.

DISCUSSION

The conclusion that GA can increase dry weight of potato plants by increasing leaf area is confirmed, but the later decline in photosynthetic efficiency of leaves sprayed with GA was apparently not because N content was less. Increasing the supply of N had little effect on N content per unit area of leaf and did not increase photosynthetic efficiency. Potato plants whose growth was increased by GA did not absorb more N even when it was freely available. One of the striking features of this and previous experiments is the similarity between GA-sprayed plants and controls at the final harvest, both in their total dry-matter content and total N content. The initial stimulating action of GA is subsequently lost, suggesting that there is a factor limiting dry matter production irrespective of leaf area. If GA were toxic in the concentrations used, dry matter production might be expected to be lower than in the controls. The limiting factor could be absorption of N by the root system, which is apparently not altered in size by GA, were it not for the fact that applying N via the leaves, and thereby short-circuiting the root system, has no effect.

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Some isolates of virus causing swollen-shoot disease of cacao in Nigeria and their interrelationships

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SUMMARY

Many symptomatically distinct virus isolates were made from cacao trees infected with swollen-shoot disease in Nigeria. The symptoms caused by typical viruses from six different localities were studied on seedling Amelonado cacao inoculated as beans. Two isolates caused swellings as the only permanent symptom and another caused only leaf chlorosis. The others caused both swellings and chloroses of different type and severity. Two atypical isolates were much less virulent than the others and caused only transient and very inconspicuous leaf symptoms.

Isolates from the same or from adjacent trees usually protected against each other, whereas those from dissimilar areas did not. This suggests that the isolates are not all closely related and they may be grouped according to the results of plant protection tests.

INTRODUCTION

Virus infection is widespread in West African cacao and several distinct viruses have been distinguished (Thresh & Tinsley, 1959). The cacao mottle leaf and cacao necrosis viruses are the least important and occur only locally, whereas the viruses which cause swollen shoot and form the subject of the present paper occur in many of the most important cacao-growing areas of Sierra Leone, Ivory Coast, Ghana and Nigeria.

The symptoms of swollen-shoot disease are not everywhere the same. Indeed, the characteristic stem swellings may be rare or even absent, and the leaf symptoms also differ between and within outbreaks. These differences are sometimes due to the host, but several strains of virus have been recognized and described from Ghana and Nigeria (Posnette, 1947*a*; Posnette & Todd, 1955). They differ not only in the type and severity of symptom they cause, but also in transmission by mealybugs (Posnette, 1950) and host range (Posnette, Robertson & Todd, 1950; Tinsley & Wharton, 1958). Avirulent strains usually protect plants against virulent ones from the same outbreak or locality, but rarely protect against viruses from elsewhere (Thresh & Tinsley, 1959, 1960).

The present paper describes the symptoms caused by different isolates of virus from outbreaks of swollen-shoot disease in Nigeria and shows that isolates can be grouped according to their ability to protect plants against each other.

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MATERIALS AND METHODS

Virus isolates

Viruses were isolated by grafting patches of bark from naturally infected trees to cacao seedlings (var. West African Amelonado) in the insectary. With a few exceptions, all the isolates described were from trees showing the commonest type of symptom in disease outbreaks within and around the areas of mass infection in Western Region (for detailed maps see Lister & Thresh, 1957).

The different isolates are described under the name of the village near which they were first collected. This convention differs from that used by Posnette (1947*a*, 1950), who referred all isolates to *Theobroma* virus I and distinguished between them by letters. This is no longer practicable, as more than seventy isolates were being studied in 1953 (Tinsley, 1953) and there are even more available now. The use of place-names is a convenient alternative, but does not imply that the virus described occurs only at the type locality, or that it is the only one to occur there.

Symptom observations

The isolates were maintained on Amelonado seedlings infected by grafts. Such seedlings did not produce uniform symptoms and mealybugs were used to infect plants as beans (Posnette, 1947*b*) for all the detailed experiments.

Symptoms changed rapidly as the leaves expanded and infected plants were examined twice each week to follow the full sequence of symptom development. This was done for at least 6 months, because distinct isolates sometimes caused similar symptoms at some stages of infection.

Protection tests

Plants used in tests of the ability of one isolate to protect against another were infected by grafting. Healthy Amelonado seedlings infected with the first virus were cut back when they had shown typical symptoms and then at least five were grafted with bark patches containing the second virus. Avirulent viruses and those causing only swellings or leaf mosaic were introduced first and later challenged with isolates causing more conspicuous symptoms. Reciprocal tests were not always possible and experiments with viruses causing similar symptoms usually gave inconclusive results.

RESULTS

The symptoms caused by isolates from different localities

1 (*a*). *Egbeda virulent strain.* The virus from this locality is the most virulent known to affect cacao in Nigeria and in early transmission work it killed some seedlings infected as beans. Transmissions from surviving plants selected a somewhat less virulent form which killed rarely, although it still caused very obvious symptoms.

The first leaves produced by inoculated beans usually showed a red banding caused by an accumulation of anthocyanins in the tissues along the primary, secondary and tertiary veins. This inconspicuous symptom was frequently associated with a distortion

and crinkling of the leaves, which sometimes became discoloured and fell. The red vein-banding disappeared as the surviving leaves hardened and showed an extensive interveinal chlorosis (Pl., Fig. 1). The plants were severely stunted and some died at this acute stage of infection. However, most plants recovered to produce leaves which were either symptomless or showed a transient and relatively mild red vein-banding, followed by vein clearing and chlorotic banding alongside the principal veins (Pl., Fig. 2). Some leaves were affected on only one side of the midrib and the distribution of symptoms then resembled the injection patterns obtained by introducing the appropriate mineral solution to shoots showing severe deficiency symptoms (Maskell, Evans & Murray, 1953). Conspicuous swellings usually appeared on the stem, hypocotyl and tap root.

Trees infected by grafts showed an indistinct transition from the severe acute to the relatively mild chronic phase of infection. However, the first leaves produced after inoculation often showed a preliminary symptom (Posnette, 1947*a*) of limited angular chlorotic flecks along portions of the third- and fourth-order veins, which sometimes became necrotic. The young leaves of the next flush then showed particularly severe symptoms and often fell before maturing, causing leafless shoots which frequently developed large terminal swellings. The pods borne on plants infected with the Egbeda virus often showed a dark-green mottle, which was sometimes the earliest symptom recorded.

1(*b*). *Egbeda intermediate strains.* Some trees in the Egbeda area showed atypical symptoms and distinctive isolates were collected from farms around the villages of Ajia and Koroboto. Infected trees at one Koroboto farm were observed monthly for 5 years and some consistently showed conspicuous leaf symptoms and numerous swellings, whereas others produced only swellings or leaf symptoms, which were sometimes mild and restricted to a few leaves. Grafts to uniform seedlings usually reproduced the field symptoms and a range of different isolates was made from adjacent trees.

1(*c*). *Egbeda avirulent strains.* The virus from Egbeda usually caused consistent symptoms in the laboratory, but occasional plants, especially those infected by single mealybugs, showed mild symptoms. Back tests showed that some of the plants contained the usual virulent strain, but others contained viruses which had consistently mild effects on seedlings, even after several transmissions by grafts or by many mealybugs.

Several of the avirulent isolates were studied and all caused a very inconspicuous transient red vein-banding, which was followed by occasional speckled clearings in the interveinal areas of a few mature leaves (Pl., Fig. 3). Some isolates also caused small root swellings.

Plants infected with avirulent isolates gave no additional symptoms when challenged with virulent viruses from Egbeda or nearby villages. By comparison, other viruses, including those from Abaku, Olanla and Ilesha, caused their usual symptoms.

2, 3. *I.N.A. farm and Ilaro.* The isolate from the Ibadan Native Administration farm was from one of the twenty infected trees in an outbreak discovered and eradicated in 1954. The virus resembled that from Bisa in Ghana (Posnette, 1947*a*), but differed from most of those collected in Nigeria in that it caused large stem swellings,

but no conspicuous or permanent leaf symptoms. Indeed, leaves showed only a very inconspicuous transient red vein-banding, sometimes followed by a slight interveinal chlorosis.

The I.N.A. virus closely resembled that from an extensive outbreak more than 60 miles away in the Ilaro area of mass infection of Abeokuta Province. Indeed, the two isolates had such similar effects that protection tests were impracticable. In other experiments, isolates from Olanla, Abaku, Ilesha, Egbeda and elsewhere caused their usual leaf symptoms in plants already infected with viruses from I.N.A. or Ilaro.

4(*a*). *Abaku.* The outbreak found near Abaku in 1957 was in a cacao area at least 4 miles from any other known infection. Isolates were made from some of the twenty infected trees and most caused inconspicuous red vein-banding, followed by an extensive interveinal chlorosis (Pl., Fig. 4). This acute phase of infection was sometimes delayed and the first leaves were then symptomless or showed only a few flecks. The subsequent flush was severely affected and sometimes the leaves and growing point became necrotic. Surviving plants recovered by producing axillary shoots with red and chlorotic vein-banding restricted to the tissues along the main veins. These symptoms were usual on plants in the chronic phase of infection, but swellings were never found.

The conspicuous symptoms of the Abaku isolates appeared after inoculating plants already infected with viruses from elsewhere. Similarly, other viruses caused swellings on plants already infected with isolates from Abaku.

4(*b*). *Abaku avirulent strains.* A few trees at Abaku showed unusually mild symptoms restricted to a few branches. Particularly avirulent strains were transmitted by single mealybugs from two of these trees. The isolates protected against virulent isolates from Abaku but not from elsewhere.

5. *Olanla.* A typical isolate from Olanla caused conspicuous transient red banding along the principal veins. These symptoms were followed by limited clearing and chlorotic banding of the tissues along and between the third- and fourth-order veins. The red vein-banding symptom became so conspicuous in later leaves as to cause a red mottle near the leaf margins, which later became an unusually intense green. Other symptoms appeared as extensive yellow interveinal specks, with translucent patches along the secondary veins of some leaves. Leaves of subsequent flushes showed the red mottle symptom, but the clearings which developed as the leaves hardened were not extensive and tended to be restricted to the tissues along the margins of the dark-green areas. Swellings appeared on plants infected for several months and the translucent areas continued to appear in some leaves.

The isolate was also studied in Ghana, where it was referred to as $1\,O_2$ (Posnette, 1950). It caused similar but more severe symptoms than the first isolate from this locality, which did not cause translucent lesions. Plants already infected with $1\,O_1$ showed no additional symptoms when inoculated with $1\,O_2$ and both isolates protected against a third from an isolated outbreak 60 miles away at Ilesha. The Olanla isolates did not protect plants against other viruses and all three isolates caused a red mottle in infected plants not already showing this symptom.

6. *Offa-Igbo.* The isolate from Offa-Igbo caused transient red vein-banding followed by yellow vein-banding and stem swellings. There was no clear distinction

into acute and chronic phases, although the vein banding in later leaves became restricted to the principal veins.

In experiments in Ghana the isolate used in these investigations protected plants from the effects of subsequent inoculation with a similar isolate from the same locality, but there was no evidence of relationship with isolates from elsewhere.

Isolates from other localities. The numerous isolates collected from other localities have been little studied. Some of them, including those from Balogun caused leaf symptoms with only occasional swellings, often restricted to the roots. Other isolates caused swellings with few obvious leaf symptoms. However, most of the isolates including those from Ife, Araromi and Ikire resembled those from Offa-Igbo and caused both swellings and leaf symptoms of various types and severities. The few protection tests gave equivocal results, but isolates from different localities seemed not to protect against each other, suggesting that they were not closely related.

Table 1. *The symptoms caused by different isolates of cacao swollen-shoot virus from various localities in Nigeria*

Isolate*	Red vein-banding	Red mottle	Inter-veinal chlorosis	Vein clearing and chlorotic banding	Trans-lucent patches on leaves	Stem swellings	Marked acute and chronic phases	Leaf crinkling and abscission	Marked stunting
Egbeda	+	−	++	++	−	++	++	++	++
Mild Egbeda	+	−	−	+	−	±	−	−	±
Koroboto	+	−	+	++	−	+	+	−	+
Ajia	+	−	+	++	−	+	+	−	+
Abaku	+	−	++	++	−	−	++	++	++
Mild Abaku	+	−	−	+	−	−	−	−	±
Offa-Igbo I	+	−	+	++	−	+	+	−	+
Offa-Igbo II	+	−	+	++	−	+	+	−	+
Olanla I	++	++	−	++	−	+	−	−	+
Olanla II	++	++	−	++	+	+	−	−	+
Ilesha	++	+	−	++	−	+	−	−	+
I.N.A.	+	−	−	±	−	+	−	−	±
Ilaro	+	−	−	±	−	+	−	−	±
Balogun	+	−	−	+	−	±	−	−	±

* The number of plus signs indicates the severity of each symptom. The sign ± indicates a mild symptom not always produced.

The relationships between the different isolates

The symptoms of swollen-shoot disease are not the same throughout Nigeria and considerable differences occur between and within outbreaks, resembling the situation in Ghana (Posnette & Todd, 1955). In each country many symptomatically distinct isolates have been obtained from different localities, outbreaks, trees and branches (Table 1).

Attempts to produce antisera have failed and plant protection tests have given results of critical importance. They enabled the different isolates to be ascribed to groups, within which there is good protection, but between which there is not. The isolates in each group are assumed to be closely related strains, they usually caused the same type of symptom and came from the same area. By comparison, the isolates in different groups often came from dissimilar areas and caused symptoms which differed in type and severity.

One interpretation of these results is that swollen-shoot disease is caused by many

unrelated viruses, each of which occurs in related strains. However, this assumption is unwarranted, because experience with viruses of other crops has shown that serologically related strains of the same virus do not always protect plants against each other (Bawden & Kassanis, 1951; Harrison, 1958).

Reference to distinct viruses with separate names is also inconvenient and gives a false impression of heterogeneity amongst isolates which have several features in common, have a similar host range, cause the same type of disease and are unusual in having mealybug vectors. For these reasons it is suggested that the different groups are major subdivisions of a single virus or virus complex. In the same way, curly-top disease of sugar beet is caused by viruses which do not interprotect; similarly with the viruses causing mosaic diseases of cassava (Storey & Nichols, 1938). Tobacco necrosis is another example of a disease which is caused by viruses that are not all closely related and some are serologically distinct, although they are not given separate names (Bawden, 1941).

In both Nigeria and Ghana each group of swollen-shoot isolates seems to be restricted to certain localities, e.g. Egbeda, Abaku and Offa-Igbo. The origin of the many different groups is obscure, but their existence has considerable practical implications. For example, an avirulent strain found to be suitable for protecting trees in one locality (Posnette & Todd, 1955) may be ineffective elsewhere. Furthermore, varieties selected from their resistance or tolerance to infection with some virus isolates may succumb to others.

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EXPLANATION OF PLATE

Fig. 1. Severe interveinal chlorosis caused by a virulent isolate from Egbeda at the acute stage of infection.

Fig. 2. Chlorotic vein banding caused by a virulent isolate from Egbeda at the chronic stage of infection.

Fig. 3. Chlorotic specks caused by an avirulent isolate from Egbeda.

Fig. 4. Severe interveinal chlorosis caused by a virulent isolate from Abaku.

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Witchweed (*Striga hermonthica*) on rain-grown pearl millet in nitrogen-deficient sandy soil of the central Sudan

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SUMMARY

Spraying with 1 lb./acre of 80% sodium 2,4-D 2 weeks after sowing reduced incidence of the phanerogamic root parasite *Striga hermonthica* (Scrophulariaceae, 'witchweed') attacking rain-grown pearl millet (*Pennisetum typhoides*) in sandy soil of the Central Sudan, but this control resulted in increased yields only on land very deficient in available nitrogen and not when ammonium sulphate fertilizer was added. Nitrogen deficiency may be more important than *Striga* attack in limiting yields of millet in these sandy soils, although in inherently fertile clay soils elsewhere in the Central Sudan *Striga* has been reported to cause heavy crop losses to sorghum (*Sorghum vulgare*) before signs of soil exhaustion appear.

INTRODUCTION

It has recently been suggested (Last, 1960) that damaging infestations of witchweed (*Striga hermonthica* Benth., Scrophulariaceae), a serious phanerogamic root parasite of grasses and grain crops in Africa, on sorghum (*Sorghum vulgare*) were associated with nitrogen deficiency of the soil, the parasite being of little importance until the crop gave at least 300% grain yield increase to 80 lb./acre of nitrogen. Dr Last's experiments were carried out on irrigated sorghum grown on alkaline clay soil at Zeidab (about 175 miles N.E. of Khartoum) and at Wad Medani (Gezira, about 100 miles S.E. of Khartoum), in fields where sorghum had been grown every year for many years without fertilizer and which had become naturally infested with *Striga hermonthica*.

In this connexion it may be of interest to describe briefly an experiment designed to investigate the effects of nitrogenous fertilizer, and of partial witchweed control by spraying with 2,4-D hormone weed-killer, on subsequent witchweed infestation and yield of rain-grown pearl millet, *Pennisetum typhoides*, on exhausted land in the Kordofan sands area of the Central Sudan (some 200 miles S.W. of Khartoum), where the soil is sandy and average annual rainfall is about 15 in. Millet is extensively grown year after year in the same field until yields become uneconomic and heavy infestations of *Striga hermonthica*, probably a strain distinct from that attacking sorghum, build up. In this experiment pre-emergence destruction of young witchweed seedlings by spraying the soil with 80% sodium 2,4-D at 1 pound per acre 2 weeks after sowing was attempted, and ammonium sulphate was applied to the sowing holes

at two rates giving 40 and 80 lb. of nitrogen per acre, respectively. Sowing was on 13 July, emergence was good and no resowing was necessary, and the millet plants were thinned to three per hole on 27 July and sprayed later on the same day. Plants in the sprayed plots began to show varying degrees of 2,4-D injury, leading to leaf shedding, 4–5 days after spraying, the damage being most marked in unmanured plots. Many affected plants had apparently recovered 2 weeks later but plant populations in the sprayed plots were appreciably reduced (Table 1).

Table 1. *Plant populations, % witchweed infestation and yield in lb./acre*

	Total number of plants		Witchweed-infested plant holes (%)				Yield (lb./acre)	
Date ...	27. viii.	5. xi.	27. viii.	10. ix.	8. x.	5. xi.	Grain	Straw
			No fertilizer					
Unsprayed	842	735	0·8	6·7	17·0	18·2	17	446
Sprayed	605	456	0·0	1·9	8·8	9·0	29	523
			40 lb./acre N					
Unsprayed	877	832	0·5	5·3	20·0	21·0	144	3034
Sprayed	756	610	0·3	2·9	13·5	13·9	134	2894
			80 lb./acre N					
Unsprayed	888	829	1·2	16·3	39·4	42·6	307	4646
Sprayed	807	701	0·6	3·3	15·5	16·3	250	4109

Witchweed plants began to appear above ground in late August and it was clear that spraying had appreciably reduced and delayed attack, as judged by percentage infested plant holes, at all three nitrogen levels. Infestation tended to increase with increasing nitrogen in both sprayed and unsprayed plots, especially at the 80 lb./acre N rate; a similar effect is reported by Last (1960) with sorghum grown on very infertile and heavily infested soil. The reduction in infestation due to spraying was reflected in increased yields of millet grain and straw only where no fertilizer had been applied, grain yield being increased by about 70% and straw yield by about 17%; even so the yield levels were very low and spraying would not be economic. In manured plots spraying reduced the percentages of witchweed-infested holes but did not give increased grain or straw yields—rather the reverse, probably due to initial phytotoxicity of the spray. Manuring, however, led to vastly improved growth and yields of both unsprayed and sprayed plots, 80 lb./acre of nitrogen increasing grain yield 18-fold in unsprayed treatments. The low available nitrogen content of this exhausted sandy soil was also apparent from an experiment in which addition of ammonium sulphate to groundnuts (*Arachis hypogaea*, Leguminosae) at planting time increased nut and fodder yields from 374 lb. (nuts) and 249 lb. (fodder) per acre in unmanured plots to 638 lb. (nuts) and 452 lb. (fodder) per acre in plots receiving 40 lb./acre of nitrogen.

In this experiment partial control of early witchweed attack was reflected in increased yields of millet on land highly deficient in available nitrogen but not when nitrogenous fertilizer was added, suggesting that the adverse effect of the parasite on yield of rain-grown millet in Kordofan sandy soil is likely to be greatest in exhausted nitrogen-deficient soil; this is in line with Last's (1960) findings with *Striga hermonthica*

attacking irrigated sorghum on alkaline clay soils farther north in the Gezira and at Zeidab.

These results suggest that nitrogen deficiency may be more important than witchweed attack in limiting yields of pearl millet in Kordofan sandy soil, i.e. soil unproductiveness results primarily from exhaustion of nitrogen rather than from heavy witchweed infestation. In the inherently fertile clay soils of marginal rainfall areas of the Central Sudan, however, fair yields of sorghum have been obtained from so-called exhausted land when witchweed attack was controlled by pre-emergence spraying of 2,4-D (Jones, 1953), and witchweed damage is reported to reach the critical level before the appearance of signs of soil exhaustion (Basinski, 1955).

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Heterodera rostochiensis (Woll. 1923) on *Solanum demissum*—a population study

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SUMMARY

Cysts of three different size grades provided three series of six different inoculum levels of *Heterodera rostochiensis* on *Solanum demissum*. The cyst numbers were adjusted so that the infestation levels in terms of eggs per gram of soil were the same in each series at any one inoculum level. Thus, the inoculum was dispersed within many small cysts, several medium cysts or few large ones. The different dispersion of the infestation had little effect on the final eelworm population. New cysts produced in the experiment were size-graded and their total egg content and hatchable larval content were estimated. Neither size-grade of the inoculum cysts nor the level of infestation had any effect on the content or hatchability of the graded new cysts. Percentage hatch appeared to decline with increase of cyst size; no useful decrease in hatching variability was achieved by using graded new cysts in hatching tests. The smallest new cysts (< 0·295 mm.) contained an average of sixty-four eggs; such cysts are easily overlooked in soil samples.

INTRODUCTION

Many factors affect the increase rate of a cyst-forming nematode. Chitwood & Feldmesser (1948), Fenwick & Reid (1953), and Schmidt (1954) have shown that the population increase of the potato-root eelworm, *Heterodera rostochiensis* Woll. is less at high than at low or medium initial infestation levels; and similar results have been obtained for other species of *Heterodera* (Goffart, 1952; Jones, 1956; Hesling, 1957). Jones (1956) suggested that this might be due to competition between larvae at high population densities. Ellenby (1954) claimed that the sex ratio of *H. rostochiensis* is influenced toward an excess of males when there is a high density of larvae in a host-plant root. The possible importance of the dispersion of the initial egg population has not been considered, however. For example, a potato-root eelworm population of 10,000 eggs/kg. of soil may be derived from fifty cysts containing an average of 200 eggs or from 200 cysts containing an average of fifty eggs. In the former the infestation is dispersed in fifty foci and in the latter in 200 foci. It is possible that numerically equal but differently dispersed eelworm populations might produce different final populations under otherwise similar conditions. To study this, and the effect of the size of the initial infestation on the final population, a pot experiment was set up in 1954 using the potato-root eelworm and a host plant, *Solanum demissum* Lindl.

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MATERIAL AND METHODS

The cyst stock used originated from one site in 1952 and was 'cultured' on potato plants in 1953. Franklin (1938) showed that the hatching of *H. rostochiensis* is influenced by the age of the cysts, so to reduce the variability of the inoculum only those cysts produced in 1953 were used. The cysts were sieved into four size grades, A, B, C and D, using sieves of 30, 40, 50 and 60 mesh/in., and the mean egg content of the cysts in the three large grades was estimated using a combination of the techniques of Fenwick (1952), Bijloo (1954), Hesling (1952) and Fidler (1956). The smallest cysts (grade D) were discarded. Three similar series of six inoculum levels were set up using cysts from one of the three size grades for each series, and the cyst numbers were adjusted so that the density of eggs was the same at any one inoculum level in each grade (see Table 1).

Table 1. *Inoculum levels in terms of eggs per gram of soil and cysts per pot*

	Inoculum level ...	1	2	3	4	5	6
	Eggs/g.	2·9	5·8	11·7	23·4	46·8	93·7
Series A	Cysts/pot (grade A cysts)	5	10	20	40	80	160
Series B	Cysts/pot (grade B cysts)	9	18	36	71	142	285
Series C	Cysts/pot (grade C cysts)	22	44	87	175	350	700

The mean egg content of cysts of each grade, estimated from examination of 200 cysts was:

Grade A 411 eggs/cyst (cyst size >0·556 mm.)
Grade B 231 eggs/cyst (cyst size 0·556–0·401 mm.)
Grade C 94 eggs/cyst (cyst size 0·401–0·295 mm.)

Each inoculum level was set up in $5\frac{1}{2}$-in. pots of dry soil (575 c.c.) consisting of a mixture of one part sand and three parts 'eelworm-free' loam (Peters, 1951). Replication was fourfold: there were seven pots containing uninoculated soil as a check that the soil was 'eelworm-free'. Each potful of soil was measured and the appropriate batch of cysts was mixed into it before it was put in a pot. When filled, the pots were placed at random in a shaded gravel plunge and watered thoroughly. One week later, on 28 June 1954, a single tuber of *Solanum demissum* was planted in each pot. The plants grew well and showed no difference whether grown in clean or inoculated soils. At the end of the growing season cysts were extracted from the pots by the technique of Fenwick (1940). The cysts from each pot were then graded similarly to the cyst inoculum, and the number of new cysts per pot was estimated as in the following example. In one pot inoculated with eighty-seven cysts of grade C, 354 cysts were found at the end of the growing season. Of these, thirty-seven were of grade A, 104 of grade B, ninety-five of grade C and eighteen of grade D. All cysts in grades A and B were larger than any of the original eighty-seven cysts, and were therefore newly produced in 1954. Of the ninety-five grade C cysts, eighty-seven were probably the

original inoculum (though some of the inoculated cysts may have been lost), so that only eight cysts in this grade were undoubtedly produced in 1954. The grade D cysts recovered were probably mostly produced in 1954, but it is possible that this grade also contained some of the inoculum cysts which, because of their stay in the soil, now passed through the 50 mesh/in. sieve. After counting, new cysts of each grade from each treatment were collected at random from a counting tray (Fenwick, 1940; Johnson, 1957) in two equal batches each not exceeding 100 cysts. The egg content of the cysts of one batch was estimated using the techniques mentioned above, and the other batch was subjected to a hatching test (Fenwick & Widdowson, 1958). Hatching of some batches was complete only after about 16 weeks. The larvae which emerged from each batch of cysts were stored in dilute formalin until counted.

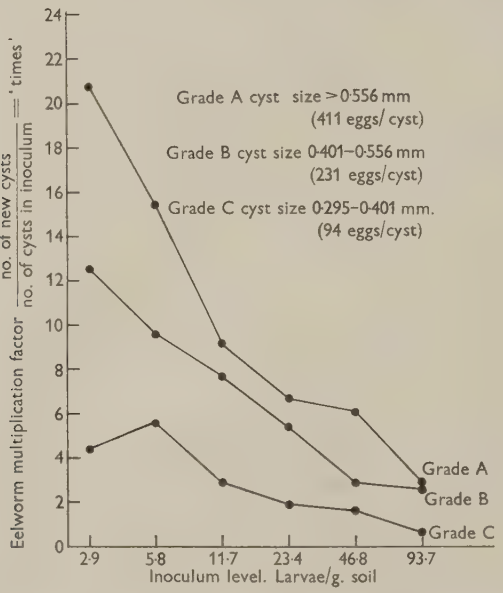

Fig. 1. Graph showing the eelworm 'multiplication-factor' derived from

$$\frac{\text{number of new eelworm cysts}}{\text{number of cysts in inoculum}}$$

for three series, A, B and C, of six different inoculum levels. For each series, cysts of one size grade only were used. The number of cysts added was adjusted so that the density of eggs per gram of soil was the same at any one inoculum level in each series. The differences between the lines reflect the number of cysts in the inoculum, demonstrating that this method of estimating eelworm increase may not be reliable.

RESULTS

The data obtained provide the following information for each infestation level in each series:

(1) Multiplication factor (based on cyst numbers).

(2) Final population (new cysts).
(3) Percentage of new cysts in each of the size grades.
(4) Hatchability of the eggs in the new cysts.
(5) 'Cyst-efficiency' (Hesling, 1957) of the host plants.
Each of the above will be considered separately.

(1) The multiplication factor with each treatment can be estimated by dividing the number of new (1954) cysts by the number of cysts in the inoculum. If this increase is plotted against the original inoculum, a curve is obtained for each of the three series of inocula (Fig. 1). It appears that the eelworm increase is greatest from the largest cysts, but this merely reflects the number of cysts in the inoculum. The true increase can only be found when the initial and newly formed eelworm populations are known in terms of eggs/pot. It is impossible to distinguish in the final population between newly formed eggs and the unhatched residue of the inoculum, so a multiplication factor based on egg numbers is unobtainable.

(2) For each of the three series, the logarithm of the number of new cysts per pot was plotted against log inoculum level (larvae per gram of soil) (Figs. 2A, B, C). Analysis of the data represented in each of these figures shows a significant linear relationship between log new cysts per pot and log inoculum level. A sample analysis is shown in Table 2.

Table 2. *Regression analysis* (*log number of new cysts per pot y on log inoculum level x*)

Series A—inoculum of grade A cysts (411 eggs/cyst)

Regression analysis

Source of variance	Sums of squares	Degrees of freedom	Mean square	*F*	*P*
Between inoculum levels					
1. Regression	1·5158	1	1·5158	49·2	0·001
2. Deviations from regression	0·0949	4	0·0237	0·769	0·20
3. Within levels (residual)	0·5549	18	0·0308	—	—
	2·1656	23			

Regression coefficient b of y on $x = 0{\cdot}48$. Standard error of b $\pm 0{\cdot}0609$.

Series B—inoculum of grade B cysts (231 eggs/cyst)

Regression coefficient b of y on $x = 0{\cdot}59$. Standard error of b $\pm 0{\cdot}0645$.

Series C—inoculum of grade C cysts (94 eggs/cyst)

Regression coefficient b of y on $x = 0{\cdot}44$. Standard error of b $\pm 0{\cdot}107$.

On the graphs (Figs. 2A, B, C) the inoculum level is on a logarithmic scale, thus the linear relationship shown indicates that rate of increase of the eelworm declines as the level of inoculum increases.

The final population for the highest inoculum rate tends on all three figures to fall away from the linear, suggesting that the maximum final population has been passed (see Hague & Hesling, 1958).

The slopes of the fitted lines of Figs. 2A, B and C are similar, and no significant difference between them could be shown by analysis, indicating that over the range of inocula used the dispersion of the initial population in few or many foci of infection had little effect on the final eelworm population.

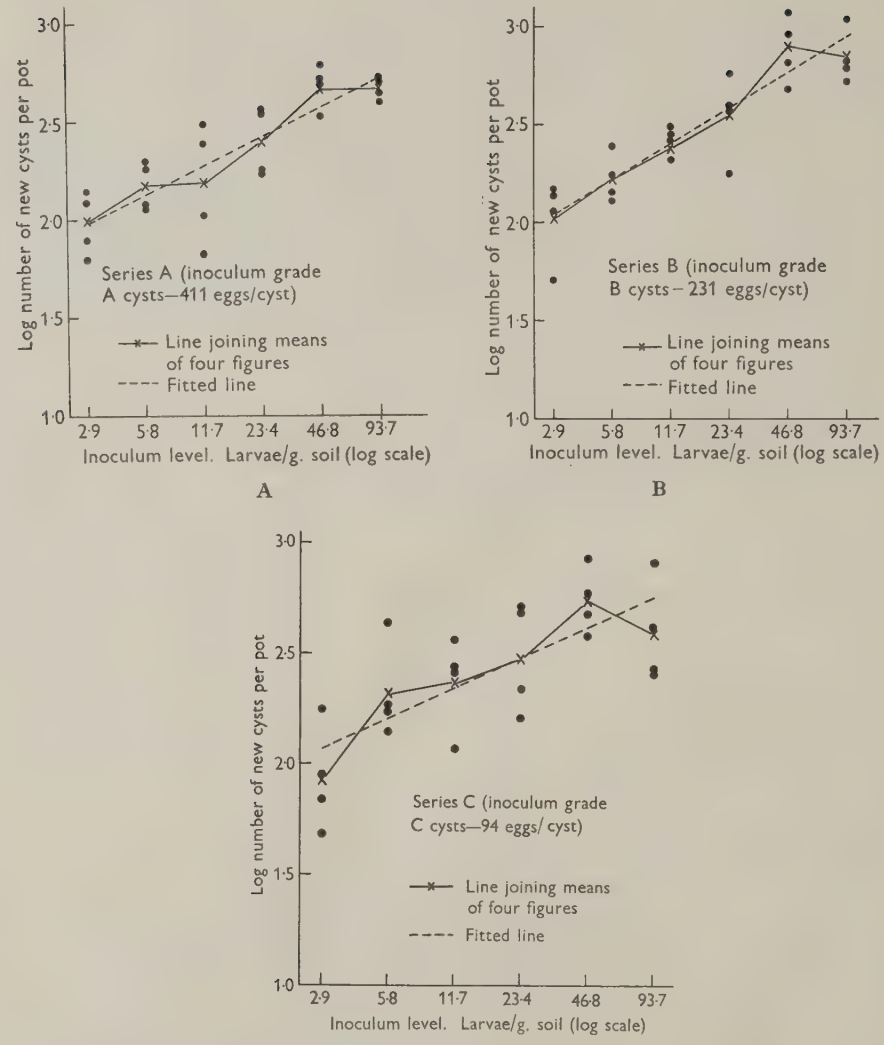

Fig. 2A. Graph showing log number of new cysts per pot produced from six different inoculum levels of *H. rostochiensis*. Cysts of large size (>0·556 mm.) with a mean content of 411 eggs were used in the inoculum.

Fig. 2B. Graph showing log number of new cysts per pot produced from six different inoculum levels of *H. rostochiensis*. Cysts in the size range 0·401–0·556 mm. with a mean content of 231 eggs per cyst were used in the inoculum.

Fig. 2C. Graph showing log number of new cysts per pot produced from six different inoculum levels of *H. rostochiensis*. Small cysts (size range 0·295–0·401 mm.) with a mean content of ninety-four eggs per cyst were used in the inoculum.

(3) If at each inoculum level within series A or B the number of new cysts in each size grade is expressed as a percentage of the total new cysts, and this percentage is plotted against the appropriate inoculum level, the resultant graphs (Figs. 3A, B) show that the inoculum level appears to have little effect on the size of the new cysts.

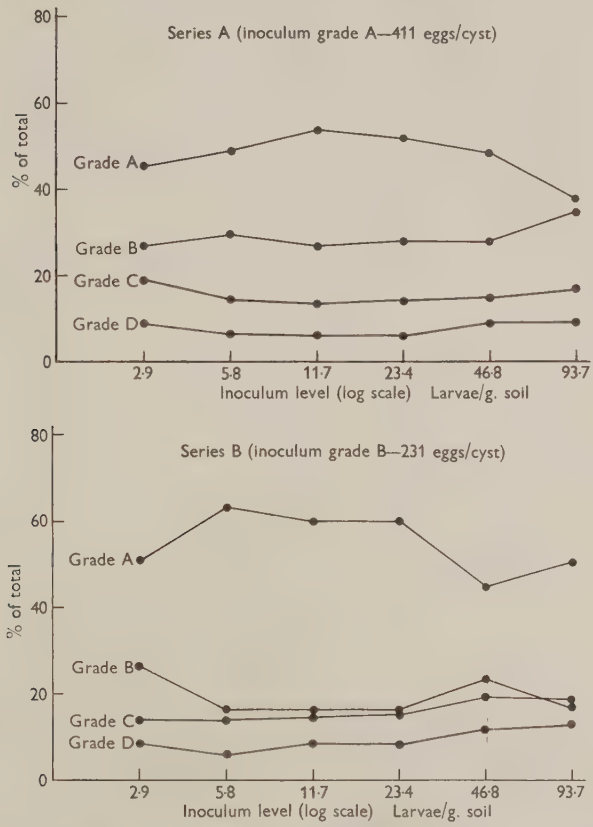

Fig. 3A. New cysts produced from each of six different inoculum levels of *H. rostochiensis* were sieved into four size grades and counted. The number of cysts in each grade was expressed as a percentage of the total and is shown in the figure. Cysts of the largest size (411 eggs/cyst) were used as inoculum.

Fig. 3B. New cysts produced from each of six different inoculum levels of *H. rostochiensis* were sieved into four size grades and counted. The number of new cysts in each grade was expressed as a percentage of the total and is shown in the figure. Cysts of size range 0·401–0·556 mm. were used as inoculum (231 eggs/cyst).

There is an indication that, from similar inoculum levels, relatively more of the largest cysts were produced in series B than in series A.

(4) Egg counts and hatching tests on batches of new cysts showed that, within each

series, the total and hatchable egg content of new cysts of the same size grade appeared to be independent of the inoculum level. The results are shown in Table 3.

Table 3 also shows that the size or number of the cysts of the original inoculum had little effect on the total or hatchable egg content of new cysts of any one size grade.

A comparison of the variability between cyst batches (standard deviation × 100/mean) in total egg content per cyst and hatchable egg content per cyst for new cysts of each grade in each series is shown in Table 4. As the individual values are derived from the examination of cysts produced from each different inoculum level in each series, they may be a little high. The variability of the total egg content per cyst and of the hatchable egg content per cyst increases with decrease of cyst size. This is to be expected for statistical reasons. The ratio between the variability of total and hatched eggs per cyst is more marked for the largest cysts which suggests that hatching is affected by cyst size, under the conditions of the hatching test.

Table 3. *The egg and hatchable larval content of new cysts*

Inoculum grade ...	Series A				Series B				Series C			
New cyst grade ...	A	B	C	D	A	B	C	D	A	B	C	D
Sieve meshes/in. ...	30	40	50	60	30	40	50	60	30	40	50	60
					(*a*) Eggs/new cyst							
Inoculum level (eggs/g.)												
(3)	*	243	117	55	492	*	150	92	512	222	*	91
(6)	*	231	113	56	481	*	101	51	506	172	*	61
(12)	*	215	112	47	504	*	123	61	456	186	*	72
(23)	*	224	94	69	480	*	145	62	467	217	*	57
(47)	*	208	111	71	429	*	113	62	490	196	*	60
(94)	*	217	121	66	462	*	125	73	413	153	*	53
Mean		223	111	61	475		126	67	474	191		66
					(*b*) Hatched larvae/new cyst							
(3)	*	165	97	63	254	*	102	75	315	181	*	75
(6)	*	173	93	47	333	*	75	45	285	174	*	50
(12)	*	158	94	57	270	*	89	53	253	197	*	66
(23)	*	151	90	62	256	*	149	38	349	139	*	56
(47)	*	200	109	63	261	*	105	60	405	108	*	77
(94)	*	185	93	63	307	*	121	71	264	155	*	50
Mean		172	96	59	280		107	57	312	159		62

* These values are omitted since new and original (inoculum) cysts are extracted on the same sieve and cannot be distinguished.

Eggs remaining in the cysts after the hatching tests were examined and counted. Eggs with dark cloudy contents, with damaged or disintegrating contents, or in which the form of the larva was indiscernable, were considered to be unhatchable and were not counted. A comparison is therefore possible between all the grades of new cysts, of the percentage of eggs hatched, the percentage of residual hatchable eggs and the estimated egg content of the cysts (determined before the hatching test). This comparison is summarized in Table 5. It appears that the percentage hatch is lower, the larger the cysts.

(5) The 'cyst efficiency' of the plants (i.e. the number of new cysts produced per pot

expressed as a percentage of the potential number of cysts assuming a 1:1 sex ratio) is shown in Table 6. The cyst efficiency is low throughout the experiment, but is within the range commonly found for this species of *Heterodera*.

Table 4. *Coefficient of variability of total egg content and hatched egg content of batches of new cysts produced from three series of six different infestation levels*

Inoculation grade ...	A		B		C	
New cyst grade	Total	Hatched	Total	Hatched	Total	Hatched
A	*	*	5·8	11·6	7·7	18·5
B	5·6	10·5	*	*	13·8	20·2
C	8·7	6·9	14·5	24·3	*	*
D	16·3	10·7	21·4	25·5	21·8	18·7

* These values are omitted because new and original (inoculum) cysts are extracted together and cannot be distinguished.

Table 5. *A comparison of the mean cyst content of all new cysts of four different size ranges, produced on* Solanum demissum

New cyst grade	Total larvae/cyst estimated w	Hatched larvae/cyst x	Hatchable larvae/cyst (residuum) y	Unhatchable larvae/cyst estimated $w-(x+y)$
A	474	296	117	61
B	207	166	26	15
C	118	101	13	5
D	64	59	5	0
Above values expressed as percentage of total larvae (w)				
A		62	25	13
B		80	13	7
C		85	11	4
D		92	8	0

Table 6. *Cyst efficiency (number of new cysts expressed as a percentage of the potential number of new cysts) of the host plants grown in three series of six different inocula*

Inoculation series ... Infestation level (eggs/g.)	A	B	C
3	10·1	11·0	9·4
6	7·5	8·4	11·5
12	4·5	6·8	6·2
23	3·3	4·8	4·2
47	3·0	5·0	3·5
94	1·5	2·2	1·3

DISCUSSION

The results of this experiment show that differences in the dispersion of the eelworm population have little effect on eelworm increase, on the egg content of new cysts or on their hatchability. Figs. 2 B and 3 B suggest that the inocula of series B had the highest potential for population increase.

Many theories may be proposed to show how the different dispersions of eelworm populations of similar density might affect eelworm increase. Thus, where eelworm cysts are small, due perhaps to adverse growing conditions during the previous season, there may be a greater proportion of 'weak' larvae, which would fail to mature, than with large cysts. Large cysts may contain so many eggs that competition for oxygen affects their hatching rate. If larvae emerge *en masse* from a large cyst, they may invade local roots so heavily that these cannot support the parasites, or more larvae may develop into males (see Ellenby, 1954; Triantaphyllou, 1960). Roots sparsely invaded from widely dispersed small cysts may be able to support the eelworms to maturity. It is possible that these effects for or against eelworm increase cancel out, or are unimportant.

Grading of the cyst inoculum at the start of the experiment and grading the new cysts at the end of the experiment are simple procedures which permit the extraction of cysts of known age. The method resembles more closely the conditions in the field than those described by Fenwick & Reid (1953) (who transplanted potatoes from infected to clean soil during the early stages of attack), and Ellenby (1955) (who kept 'inoculum' cysts in gauze baskets placed in pots of potato plants).

In spite of size grading, the variability of the total and hatched egg content of the batches of new cysts was high, and of the order found by Fenwick (1949). The higher variability of the number of hatched eggs from the largest cysts may depend not only on their 'inherent' properties (e.g. immature egg content) but also on differences in the physical or chemical environment of the eggs, produced by their numbers. For example, there are fewer individual larvae in a batch of 100 small cysts than in a batch of 100 large cysts whose respiratory requirements may not be met by the available oxygen supply.

Knowledge of the relationship between the number of new cysts per pot and the initial infestation level may provide a practical method of estimating the kill of *H. rostochiensis* after treatments with, for example, different concentrations of a nematicide. When the size of the final population produced by treated cysts on host plants is alone used as a criterion of eelworm kill, the effect on the population of the higher increase of the eelworm from low levels of infestation is not taken into account. The final population must first be related to the initial population which produced it, for it is the viability of this initial population which gives a measure of treatment effect. This can be done if the form of the curve (e.g. Fig. 2) is known and is compared with a curve of final population plotted against treatment (e.g. nematicide concentration). The highest initial eelworm population used should not exceed that which will give the maximum final population, for initial populations higher than this may result in a final population lower than the maximum (Hague & Hesling, 1958).

Counts of graded cysts from pots inoculated with grade C cysts showed that there was some loss of the inoculum cysts because the number of cysts of this grade extracted from each pot was frequently less than the number of cysts used in the inoculum. This loss may have happened because small cysts may decay more rapidly than large or because they may be more difficult to recover after several months in soil.

The range of the total egg content of the new cysts was between 512 and 47 eggs per

cyst, and the range of the hatched egg content between 405 and forty-seven eggs per cyst. In this experiment every effort was made to collect all cysts from each pot, and it is noteworthy that cysts of the smallest grade, which passed through a mesh of aperture 0·295 mm. square, contained an average of sixty-four eggs. Cysts of this size can easily be overlooked.

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The list of speakers and titles of papers given at Meetings of the Association in 1960 are noted in the Report of the Council on p. 387.

Meetings were held in the Natural History Museum on 20 January 1961 (symposium on recent developments in plant nematology) and on 10 February (symposium on recent work in plant virus diseases).

Abridged versions of the following papers are presented below:

Meeting of 23 September 1960

Life history and bionomics of the vetch-leaf gall midge. By Messrs R. Gair and C. T. Guile.

Meeting of 11 November 1960

The effect of γ-radiation on some wood-boring insects. By Mr J. D. Bletchly.
Studies on the use of radiation in oat breeding. By Mr T. D. Johnston.

Meeting of 9 December 1960

Induced tolerance of stored-product beetles to methyl bromide. By Messrs H. A. U. Monro, A. J. Musgrave and E. Upitis.

A study of the development of beetle infestations in flour-milling machinery. By Mr C. E. Dyte.

Meeting of 20 January 1961

The importance of plant-parasitic nematodes in Britain. By Dr H. C. Gough.
Practical problems and recent trends in nematode control. By Mr F. C. Peacock.

Meeting of 10 February 1961

Viruses isolated from cherry trees with rasp-leaf and leaf-roll diseases. By Mr R. Cropley.

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Life history and bionomics of the vetch-leaf gall midge

By R. Gair and C. T. Guile

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In 1953 and subsequent years winter tares (*Vicia sativa* L.) grown for seed in the East Midlands were found heavily infested with larvae of the vetch-leaf gall midge, *Dasyneura viciae* (Kieffer), and an earlier paper (Gair & Guile, 1956) reported preliminary observations on the biology and chemical control of this pest. The present paper presents a summary of further investigations into its life history, bionomics and plant host range.

Observations were conducted partly on infested tare crops in North Lindsey and partly in an unheated insectary at Shardlow Hall, Derbyshire. Breeding jars of the type used by Barnes (1946) served adequately for collecting data on the emergence of adult midges and their para-

sites. Vetch plants were propagated from seed in an unheated glasshouse and the seedlings transferred to large pots enclosed in muslin cages for transference tests. The results refer chiefly to the years 1956 and 1957.

As the original description of the midge (Kieffer, 1888) was rather inadequate and as Kieffer left no type material, the following notes on certain stages of its life history are provided in amplification.

The egg is smooth, shiny and yellow-white when newly laid, ellipsoidal, 0·30 × 0·08 mm. (mean of twenty eggs). After 3–5 days the contents become hyaline and a central red spot, the ventriculus, appears with the fat body becoming differentiated as ten white patches on either side of it. The incubation period lasts 5–7 days.

The larva is almost transparent at eclosion but later becomes orange. The sternal spatula is bilobed anteriorly, tapering posteriorly to end in a globular region. The mature larva measures 2·25 × 0·66 mm. (mean of twenty specimens). During May and June the larva takes 20–25 days to reach maturity. Its movement from plant gall to the soil usually occurs during showery weather.

The cocoon, 1·92 × 0·78 mm. (mean of twenty specimens), is tough, silken and ellipsoidal, with a straw ground colour but often partially coated with soil particles.

The puparium is fusiform.

The adult midges are typical members of the genus *Dasyneura*. They fly actively in warm, sunny weather. Copulation has been observed both in the field and laboratory and lasts from 10 sec. to more than 1 min. Several males may cohabit with the same female. At oviposition the gravid female midge excitedly investigates the growing points of susceptible vetch species, probing the plant with her long ovipositor. Supporting herself with the first two pairs of legs, she curls the abdomen forward under the thorax and deposits eggs singly or in groups of up to fifteen within folded leaflets at the growing point or occasionally in leaflets some distance from it. Gall formation commences some 12–20 days after oviposition and is usually well advanced 7–10 days later.

The numbers of adult midges and their chief hymenopterous parasite, *Prosactogaster demades* (Wlk.), emerging from galled plant material in breeding jars was recorded in detail during the years 1956 and 1957. In 1956 first-generation midges began to emerge from overwintering cocoons on 16 May and reached their peak in emergence numbers by early June. Egg laying coincided with this period in tare fields in North Lindsey. A sharp drop in emergence numbers occurred after mid-June, at which time large numbers of parasites appeared. Second-generation midges began to emerge in late June and reached their peak by mid-July. Parasites of this generation reached their peak period of emergence during late July and early August. A partial third generation of midges began emerging in early August and was followed in September by the emergence of large numbers of the parasite.

In 1957 the emergence pattern differed appreciably, for very few midges appeared in the second generation and none in the third. There was some overlapping of generations in both years and the sex ratio remained fairly constant at 40:60 (♀♀:♂♂). No evidence was forthcoming for the existence of unisexual families in *Dasyneura viciae*.

Not all the larvae maturing in the late summer become adults the following year, a proportion emerging during the second, third or even fourth successive years. This phenomenon of delayed emergence has been noted in other gall midges including *Contarinia pisi* (Winn.) (Barnes & Arnold, 1960). Normally the larvae of *Dasyneura viciae* fall from their galls into the soil and there spin cocoons, but under insectary conditions at Shardlow they often remain quite naked throughout the winter and metamorphose directly into puparia.

Work on the host plant preference range of *D. viciae* involved (*a*) the exposure to natural infestation of small field plots of various vetches and (*b*) the introduction of one or more gravid females into muslin cages containing one or more vetch species. The criterion of successful colonization of the plant was taken as the development of galled leaflets containing mature larvae. As a result of these experiments we propose a classification of the vetch species examined into four groups depending on their susceptibility to attack by the midge.

Group I vetches are highly acceptable to the female midge for oviposition and are preferred above all other species tested. They include *Vicia sativa* (winter and spring tares) and *V. sativa* var. *alba*.

Group II vetches are moderately acceptable to the female midge in the presence of Group I vetches and include: *V. angustifolia, V. calcarata, V. grandiflora, V. hybrida, V. peregrina, V. picta* and *V. segetalis.*

Group III vetches, which are acceptable only in the absence of vetches in Groups I and II, are *V. atropurpurea, V. pannonica, V. villosa* and *Vicia* sp. (Polyploid spring tare).

Group IV vetches are completely unacceptable to the female midge for oviposition purposes. This group includes the majority of the species tested, namely *Anthyllis vulneraria, Vicia articulata, V. biennis, V. biflora, V. bithynica, V. cassubica, V. cracca, V. dasycarpa, V. dumetorum, V. eriocarpa, V. erviformis, V. ervilia, V. faba, V. fulgens, V. gigantea, V. globosa, V. graminea, V. hirsuta, V. lathyroides, V. lutea, V. narbonensis, V. oroboides, V. orobus, V. pisiformis, V. sylvatica, V. tenuifolia, V. tetrasperma* and *V. unijuga.*

The identity of certain of the above species, particularly *V. calcarata, hybrida, peregrina, picta* and *lathyroides* is somewhat doubtful. Those species with a large, compact growing point are usually accepted by the midge, while vetches having hairy or finely divided leaflets are not generally favoured for oviposition.

Although *Vicia cracca* is commonly supposed to be a host of *Dasyneura viciae*, in the experiments described above it was completely unacceptable to the midge. In August 1956 galled leaflets were taken from wild, infested *V. cracca* plants near Shardlow and from this material eggs, larvae and adults of a gall midge were bred in the laboratory. Eggs of this '*cracca*' midge are red, 0·26 × 0·08 mm. (mean of six eggs). The larva takes its predominantly orange colour from the contents of the alimentary canal, while both ends of the body are suffused with pink. The gall consists of a leaflet swollen along the midrib and later folding into a pod-like green structure usually containing one mature larva. The adult midges are bright cherry-red in colour and are distinctly larger than *Dasyneura viciae*; they bear some semblance to the description of *D. loewiana* Rübsaamen.

Cherry-red midges were also reared from galled leaflets of *Vicia tetrasperma* collected in North Lindsey and appear closely similar to the '*cracca*' midge. Under laboratory conditions these midges from *V. cracca* and *V. tetrasperma* failed to produce galls on *V. sativa.*

In years of abundance such as 1953, 1954 and 1955 *Dasyneura viciae* can seriously affect production of seed tares. Its overall importance as a pest is not great, however, as the total acreage of winter tares in the East Midlands rarely exceeds 3000 acres, much of which is cut green or grazed by sheep and therefore not subject to midge attack.

We are indebted to the late Dr H. F. Barnes for his constant help and encouragement.

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The effect of γ-radiation on some wood-boring insects

By J. D. Bletchly

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Eradication of wood-boring insect infestations (for example, deep-seated attack by the deathwatch beetle, *Xestobium rufovillosum* Deg.) frequently presents special problems, particularly in large-dimensioned timbers where restricted access to all surfaces renders treatment difficult. Normally insecticidal fluids or poisonous gases are used, but their value is often restricted by

the relative impenetrability of some timbers or by the difficulty of obtaining adequate sealing as well as other factors. Recent investigations into the possibilities of using various types of radiation for insect control have been reviewed by Hassett (1956) and Fisher (1958). Application of high-frequency fields is limited by the absence of a differential heating effect on insects (Thomas & White, 1959; Thomas, 1960). Very high temperatures are required to achieve complete insect mortality by using infra-red heating (Fisher, 1958; For. Prod. Res. 1959).

γ-Radiation appeared to offer possibilities of treating large timbers. Limited data were already available for *Lyctus planicollis* Lec. (Hassett & Jenkins, 1952) and these served as a useful basis for further investigations. Owing to difficulties in breeding death-watch beetles, initial studies were concentrated on another powder-post beetle (*L. brunneus* Steph.), which can be cultured under controlled conditions of temperature and humidity, thus providing all stages of the life-cycle throughout the year (Harris & Taylor, 1960). In this way data on the order of dosages required to prevent completion of development were obtained and these were subsequently utilized in further studies on the common furniture beetle (*Anobium punctatum* Deg.), on the death-watch beetle and to a small extent on the house longhorn beetle (*Hylotrupes bajulus* L.). Progress reports on these investigations have been published (Bletchly, 1958; Bletchly & Fisher, 1957; For. Prod. Res. 1955–59).

In this summary of the data so far obtained only broad results are given and statistical analyses have been omitted. In many instances, apparent lack of effective control at certain dosages represents only very limited survival, successful development or fertility. It is intended to publish full experimental data in due course when these studies have been completed.

Some of the literature on the effects of X- and γ-rays on certain insect pests in grain and soil or of veterinary importance has been reviewed by Cornwell & Morris (1959).

TECHNIQUE

Studies have been made on the effects of γ-radiation on eggs, larvae, pupae and adults. Oviposition was obtained on suitable egg-laying blocks—oak sapwood veneers for *Lyctus* (Bletchly, 1960), Corsican or Scots pine sapwood blocks with muslin surfaces for *Anobium* (Bletchly, 1952) and decayed oak sapwood for *Xestobium*. Larvae were either irradiated free (*Lyctus*) or within oak sapwood blocks (*Lyctus*), or pine (*Anobium*) or decayed oak sapwood (*Xestobium*). Pupae were treated free (*Anobium*) or within decayed oak sapwood (*Xestobium*); adults were irradiated free or within infested wood. For studies on subsequent fertility and viability of eggs, treated pupae and immature and mature adults were provided with egg-laying blocks. In addition naturally infested material has been treated. Cobalt-60 sources giving dose rates from 50 to 1300 r./min. have been used as well as higher rates: all dosages are expressed in röntgens.

EFFECTS OF γ-RADIATION ON THE DEVELOPMENTAL STAGES

Possible lines of research considered were the results of γ-ray treatments in: (*a*) effecting rapid mortality, (*b*) preventing completion of development or causing sterility in adults, or (*c*) producing and releasing sterile adults in quantities sufficient to ensure a high proportion of abortive matings, cf. control of the screw-worm (*Callitroga hominovora* (Cqrl.)) (Bushland & Hopkins, 1953; Lindquist, 1955; Borstel, 1960). The last method was not pursued owing to absence of suitable techniques for culturing *Anobium* and *Xestobium* and because other satisfactory methods of controlling *Lyctus* infestations are available.

Initial studies showed that high dosages were needed to kill *Lyctus* larvae in a short time (Table 1) or to prevent emergence of beetles ($>$ 72,000 r.) when the adult stage had been reached in the experimental samples. Since such dosages would involve severe hazards if applied in practice, the effects of lower dosages on (*b*) above were investigated. For this purpose small samples, generally containing one stage only of the life cycle, or larger portions of naturally infested material containing one or more stages in the insect's development were employed.

For the *Lyctus* investigations culture material was normally used. To test whether the susceptibility of such insects differed from that of a natural population, larvae of the latter were

irradiated at 4000, 6000 and 8000 r.; emergence occurred only from material treated at 4000 r. indicating a similar order of resistance to that found in the cultured larvae.

Eggs. Dosages required to prevent eggs hatching depended on the stage of development reached, newly laid eggs being much less resistant than older ones (Table 2). Most of these experiments were conducted under outdoor conditions. The incubation periods of the untreated controls were not always determined concurrently and it is, therefore, impossible in such instances to gauge accurately the stage of development reached by the eggs at the time of treatment: this probably accounts for the anomaly apparent in the results for the 13 to 15-day-old *Anobium* eggs. In the case of *Anobium* average incubation periods obtained in other years are given. Where a small proportion of newly laid eggs hatched following irradiation, larvae survived for considerable periods at least, but it is not known whether they would have com-

Table 1. *Effect of γ-radiation on survival of free larvae of* Lyctus brunneus

Dosage (thousands röntgens)	Initial numbers of larvae	Numbers of larvae surviving after weeks															
		1	2	3	4	5	6	7	8	9	10	11	12	13	17	20	24
Untreated controls	23	22	22	22	21	19	17	16	16	14	13	13	13	13	10	7	1
8	21	20	19	18	17	12	10	8	5	5	1	—	—	—	—	—	—
16	21	21	18	15	14	7	3	2	2	2	1	—	—	—	—	—	—
32	21	21	18	15	11	7	2	1	1	1	—	—	—	—	—	—	—
48	20	20	17	17	17	12	7	4	4	3	—	—	—	—	—	—	—
72	22	18	14	11	9	2	1	1	—	—	—	—	—	—	—	—	—
100	20	17	14	7	5	3	1	1	—	—	—	—	—	—	—	—	—
150	24	23	12	4	1	—	—	—	—	—	—	—	—	—	—	—	—

Table 2. *Effect of γ-radiation on hatching of eggs (outdoor conditions)*

Species	Average incubation period (days)	Age of eggs at time of treatment (days)	Dosage which prevented 95% of eggs hatching (thousands röntgens)		Dosage which prevented larval survival (thousands röntgens)		Time between treatment and examination (months)	Year irradiated
			Not effective	Effective	Not effective	Effective		
Anobium punctatum	*	1–4	1·5	2	—	—	—	1956, 1957
		3–5	—	4	—	—	—	1957
		13–15	—	4	—	—	—	1956
		13–15	8	—	—	—	—	1957
	27†	14–21	—	6	—	—	—	1959
	*	21–26	64	—	—	—	—	1956
		24–26	96	—	—	—	—	1957
Xestobium rufovillosum	36	2–4	—	1	1	2	7	1956
	39	5–8	1	2	—	—	—	1955
	36	10–13	2	4	—	—	—	1956
	?	23–26	24	32	8	16	7	1956
	40	26–30	—	100	—	—	—	1958
	40	27–33	32	—	8	16	7	1956
	?	32	80	—	—	—	—	1957
	36	31–33	—	100	—	10	8	1959
Hylotrupes bajulus	16†	5–7	—	6	—	6	—	1959
	16†	7–11	10	12	—	6	7	1959

* Average incubation periods outdoors from 30 to 39 days.
† Laboratory conditions.

pleted their development. Although high dosages were required to prevent more mature eggs hatching, larvae only survived when such eggs were treated at lower dosages.

When *Anobium* eggs (following irradiation) were stored in the laboratory during the warm summer of 1959 lower dosages prevented hatching compared with those used in other years in which eggs of a comparable stage of development were kept outdoors. Warm conditions thus appeared to have an adjuvant effect in increasing the potency of γ-radiation in a similar way to that found by Baldwin & Narraway (1957) in the case of X-rays. An investigation into this possibility was undertaken with 4-day-old *Lyctus* eggs which had been kept at 25° C. and 75 % R.H.; under these conditions the normal incubation period is 6–7 days. Following irradiation, the eggs were stored at a range of temperatures but at constant humidities of 75 % R.H. In general lower dosages prevented hatching when eggs were stored at the higher temperatures following treatment (Fig. 1).

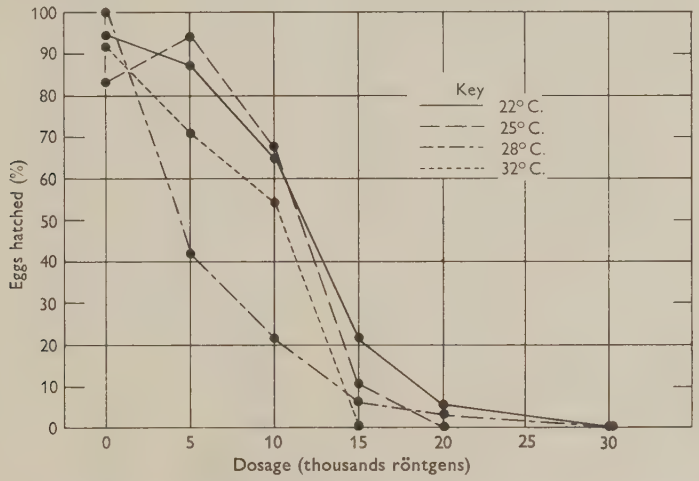

Fig. 1. Effect of temperature on hatching of eggs of *Lyctus brunneus* after irradiation (R.H. 75 %). Note: due to disintegration of samples gross egg counts for untreated controls for 25 and 28° C. were not available.

Larvae. Higher dosages were required to prevent completion of development of older *Lyctus* larvae than younger ones (Table 3). Irradiation of *Lyctus* and *Xestobium* larvae at 4000 r. produced a proportion of adults with deformed elytra. A current experiment involving irradiation of *Lyctus* larvae at 4000 r. indicates greater variation in the weights of the resultant adults than in the untreated controls. Death following irradiation usually occurred during moulting (possibly the final moult before pupation) or during the pupal stage, as previously observed in *Ephestia* (Whiting, 1950). The time of treatment has been given in Table 3, since this is of importance in the case of naturally infested material studied under outdoor conditions: for example, treatments during summer of material naturally infested by *Anobium* would affect larvae likely to give rise to adults the following or in subsequent years, since pupation occurs chiefly in May and June. Further, *Xestobium* material similarly infested would contain both larvae and adults through much of the year owing to the long over-wintering period of the adults (Fisher, 1938); in consequence when treatments were given during the winter the effect on completion of larval development would only be apparent with the adult emergence of the spring next but one.

Studies are in progress on the possibility of inheritance of sterility in subsequent generations of *Lyctus* following irradiation of larvae at 4000 r. Preliminary investigations suggested that this did not occur at any rate on an appreciable scale.

Table 3. *Effect of γ-radiation on larvae (outdoor conditions)*

Species	Material naturally infested (N.I.) or prepared by larval transfer (L.T.)	Stage of development at treatment	Dosage which killed larvae (thousands röntgens)			Dosage which prevented adult emergence (thousands röntgens)		Viability of eggs (E) or larvae (L) (F. 1 generation) after dosages (thousands röntgens)		When treated
			Not effective	Effective	Time between treatment and examination	Not effective	Effective	Not effective	Effective	
Lyctus brunneus	N.I.	1–3 days	—	—	—	4	6	—	—	(A)
	N.I.	⅛ to ½ grown	—	—	—	6	8	(L) 4	—	(A)
	N.I.	Large	—	—	—	6	8	(L) 2	4	(A)
Anobium punctatum	L.T.	Various	10	—	27 months	6	8 (B)	(E) 4	6	May 1956
	(C) N.I.	21 months	8	—	6 months	Material cut up		—	—	March 1959
	N.I.	Various	—	—	—	6	8	—	—	June 1957
	N.I.	Various	—	—	—	—	8	—	—	March 1958
	N.I.	Various	—	—	—	—	8	—	—	July 1959
Xestobium rufovillosum	L.T.	Large	6	—	3 years	2	6 (B)	(L) 2	—	May 1956
	L.T.	Various	—	6	2 years	—	6	—	—	Spring 1957
	L.T.	Medium and large	Material not cut up		—	4	6	(L) 4	—	May 1958
	(C) N.I.	9 months	8	—	6 months	Material cut up		—	—	March 1959
	N.I.	Various	—	6	22 months	—	5·8 to 6·5 (B)	—	—	January 1957
	N.I.	Various	Material not cut up		—	10 (D)	—	(L) 10 (D)	—	March 1958

(A) Controlled conditions 25° C. 75 % R.H.
(B) No emergence before material cut up.
(C) Egg-laying blocks.
(D) Three sterile adults emerged 1959, none 1960.

Pupae. Few data are at present available on the effects of γ-radiation on this stage but the following information has been obtained by irradiating both sexes of *Xestobium* pupae and mating the resultant adults together.

Dosage (thousands röntgens)	Observation
12·0	Development completed
6·0, 10·0 and 12·0	Numbers of eggs laid reduced
6·0	Hatching occurred
10·0	No hatching occurred

Adults. Data were obtained on (*a*) *Lyctus*—freely emerged adults, (*b*) *Anobium*—immature specimens reared from pupae or freely emerged mature beetles and (*c*), by my colleague, E. C. Harris, on *Xestobium*—immature or mature beetles being cut out from naturally infested willow at different times from early winter to late spring, as well as others *in situ* in naturally infested elm and willow (Table 4). Pre-irradiation matings could only have taken place in the case of naturally emerged mature beetles: in other instances matings were subsequent to treatment.

Table 4. *Effects of γ-radiation on adults (outdoor conditions)*

Species	Type of mating*		Dosage which prevented eggs hatching (thousands röntgens)		Eggs hatched (%)	When irradiated
	Males	Females	Not effective	Effective		
Lyctus brunneus	M T	M T	4	6	—	(A)
	M T	M UT	8	—	—	
	M UT	M T	8	—	—	
Anobium punctatum	M T	M T	6	8	—	1957
	IM T	M UT	8	—	21·7	1957
	M UT	IM T	8	10	—	1957
Xestobium rufovillosum	M T	M T	4	8	—	May 1956
	M T	M T	—	10	—	Mar. 1958, 1959
	IM T	IM T	8	10	—	Dec. 1956, Oct. 1957
	IM T	IM UT	10	—	2·8	Dec. 1956
	IM T	IM UT	15	—	0·8	Oct. 1957
	M T	M UT	—	15	—	Mar. 1959
	IM UT	IM T	10	—	9·1	Dec. 1956
	IM UT	IM T	15	—	1·0	Oct. 1957
	M UT	IM T	15	—	0·5	Mar. 1959
	M UT	M T	20	—	0·2	Mar. 1959

* Beetles mature (M), beetles immature (IM), beetles treated (T), beetles untreated (UT)

(A) Controlled conditions 25° C., 75 % R.H.

A higher dosage was necessary to prevent eggs hatching when an individual of one sex was irradiated and mated to an untreated individual whichever way round this might be, than if both sexes were irradiated and mated together. This is probably due to incomplete sterilization of all the gametocytes. Thus if a treated individual is mated to an untreated individual the reduced numbers of effective gametes in the former have a much better chance of forming a fertile zygote than where two affected individuals are mated together. According to this argument, if a sufficient number of individuals treated at dosages higher than those giving apparent sterility are mated together, fertile zygotes should occasionally result although much less frequently than when only one sex is treated. Thus additional studies have shown that the deduction made in an earlier paper concerning irradiation of *Lyctus* adults is not correct (Bletchly, 1958).

The age of the adult at the time of treatment is likely to be important only for *Xestobium* since both *Anobium* and *Lyctus* adults are comparatively short lived. Irradiations of *Xestobium*

adults at different times in the pre-emergence period, when the sex organs are developing, showed no marked difference in resistance judged on the basis of egg viability, from which it also follows that there is no obvious recovery after young adults are irradiated. Although these conclusions differ from those of Cornwell & Morris (1959), it is pointed out that only a limited range of somewhat widely spaced dosages was tested. No consistent difference was found between the length of life of treated and untreated *Xestobium* adults.

When both sexes of *Xestobium* were irradiated and mated together, varying results were obtained on the total oviposition; at dosages as high as 15,000 r., the numbers of eggs laid by treated and untreated beetles were similar, but at lower dosages more were sometimes laid by the treated individuals. When eggs laid by irradiated *Lyctus* and *Xestobium* adults were viable, the larvae often lived for several months. However, further studies are needed on the long-term development of such larvae.

EFFECT OF LOW DOSE RATES

A small-scale experiment with transferred *Anobium* larvae to compare the effects of irradiation at 50 r./min. and 1185 r./min. indicated little if any difference. A larger experiment with one-half to two-thirds grown larvae from *Lyctus* culture material resulted in emergence following irradiation at 6000 r. but none after 8000 r. whether treated at 50 or 1085 r./min. (the source having decayed by 100 r.).

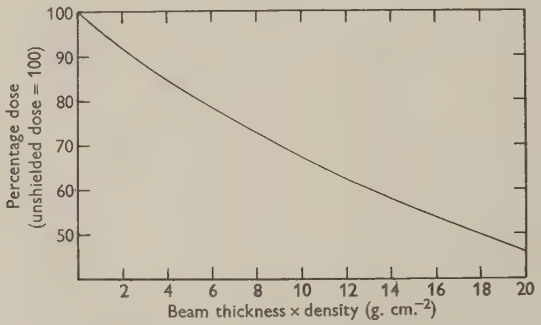

Fig. 2. Absorption of γ-rays in sectional Scots pine block.

ATTENUATION OF γ-RAYS IN WOOD

Lakatosh (1956) and Badun (1959) showed that penetration of γ-rays in wood depends on its density, moisture content and thickness, but with hard γ-rays the direction of the grain was unimportant. Studies have also been carried out by Parrish (1956) and Parrish & Myser (1956, 1957). Murray (1959) concluded that on the basis of cost, γ-ray treatments of packaged goods in bulk is practicable provided the handling is rapid and efficient.

Data on attenuation have been obtained at the Atomic Energy Research Establishment, Wantage, on a sectional four-sided block of Scots pine 2 ft. thick made up from 1-in. boards in such a way that they could be separated to allow attenuation to be measured through a range of thicknesses (Fig. 2). Using a cobalt-60 source it was shown that attenuation differed with the geometry of the beam. With a 90° solid angle, which appeared to be the most practicable, the intensity of irradiation at the fringe was about half that at the centre. This difficulty could be overcome by giving overlapping treatments. At greater angles, fringe radiation decreases and, although a greater coverage of wood surface could be obtained, there would be greater risk of some of the radiation passing above and below the timber thus increasing the hazards as well as being uneconomic. An attenuation of the order of 50 % occurred in an approximately 40-cm. thickness of this block: the relationship between timber thickness and the percentage dose received was roughly linear. Data have been obtained on the length of time of treatment, which depends on the distance of the source from the timber and on its thickness. A 1000-c. source of cobalt-60 would require about 1 ton of lead to provide about 8 in. of back-shielding

and about 6 in. elsewhere to prevent scattered radiation in other directions. In the absence of such shielding, it would be necessary to evacuate all points within about 1 mile in the line of sight of such a source. Such a weight of shielding presents formidable engineering problems with regard to the need for a mobile source to treat *Xestobium*-infested timbers *in situ* in the roofs of ecclesiastical and other buildings, operating from floors not designed to carry heavy loads. The time of treatment is an important economic factor: for example, a source such as the above, 13 in. from a timber 12 in. thick and of density 0·8 would take 6 hr. to treat a linear foot. Electron accelerators would present easier problems regarding scanning and shielding but are comparatively inefficient as sources of γ-radiation.

CONCLUSIONS

Sufficient data are available to show that γ-radiation is a possible method of controlling wood-boring insect infestations when treatments are carried out at dosages adequate to prevent completion of development or to produce sterile adults. It appears that a dosage of about 10,000 r. would be needed, although further information is required on the survival of larvae hatching from irradiated eggs. An additional safety margin would be desirable plus a further correction to allow for attenuation in the timber. The latter would depend on the thickness and density of the wood. The higher fertility exhibited when an individual of one sex is irradiated and mated to an untreated beetle of the opposite sex is somewhat academic, since both sexes would normally be irradiated simultaneously. Nevertheless, when 100 % control is not achieved, this factor represents an additional risk in the possibility of re-infestation of susceptible wood since γ-radiation at such dosages is thought unlikely to have any effects on the nutritional value of timber. The use of radio-active substances for the dual role of eradication and preservation has been considered by Sandermann & Casten (1956) and rejected owing to the hazards involved. It appears that the mechanical difficulties of providing adequate shielding for a radio-active source preclude the use of γ-radiation for controlling wood-boring insect infestations in timbers *in situ* in most instances.

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Studies on the use of radiation in oat breeding

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The ability of ionizing radiations to cause heritable changes in animals and plants was first discovered about 30 years ago by Muller (1927) and Stadler (1928) working in America. Since that time a large amount of research has been carried out on this phenomenon and the production of numerous useful mutants of crop plants by irradiation of existing varieties has been reported from Sweden (MacKey, 1956) and elsewhere. Little research has been conducted, however, on the practical considerations involved in using mutagenic techniques in crop improvement, compared with classical breeding methods.

The investigation now described was designed to give information on the problems involved in the routine use of a seed irradiation technique for the production of improved oats, and to compare the technique with the method of hybridization and pedigree selection as at present employed at the Welsh Plant Breeding Station. The investigation was continued over five generations, at the end of which all promising selections were put into variety trials to be tested against currently grown varieties and advanced generation selections from the breeding nursery. During all generations the scale of the investigation was kept within the limits anticipated to apply if the technique were adopted as routine in place of, or in conjunction with, other breeding methods.

The varieties selected for study were S. 147 and S. 172 winter oats. Both were bred at the Welsh Plant Breeding Station and were known to be of high uniformity and purity of seed.

S. 147 was subsequently studied in greater detail and the following remarks refer only to that variety.

Two lots of dry seed of S. 147 were exposed to X-rays to receive 20,000 and 30,000 r., respectively. Subsequent to irradiation these two lots were treated as a single unit to avoid bias in favour of either during the selection procedures applied. The irradiated seed was sown in the greenhouse on sterilized soil and planted into the field in isolation from other oats to avoid contamination by pollen which is known to occur readily due to the disturbances caused in X_1 generation plants by irradiation (Caldecott, Harland & Roberts, 1959). Seed was collected from each plant and sown in the following autumn into a total of 600 progeny rows.

At this stage some of these rows can be assumed to be segregating for irradiation-induced mutations, as indeed could be observed by the frequent appearance of chlorophyll-deficient types. However, from previous experience with the oat crop it was not considered worthwhile at this stage to attempt selection of small deviations from the normal in continuously variable characters, the isolation of which was the main purpose of the investigation. Similarly the X_2 generation was not space-planted because the performance of oats under such conditions is not a reliable guide to performance under field conditions. Therefore ten panicles were taken at random from each row and grown in the X_3 generation as single-panicle progeny rows, making a total of 6000 rows occupying an area of 0·6 acre.

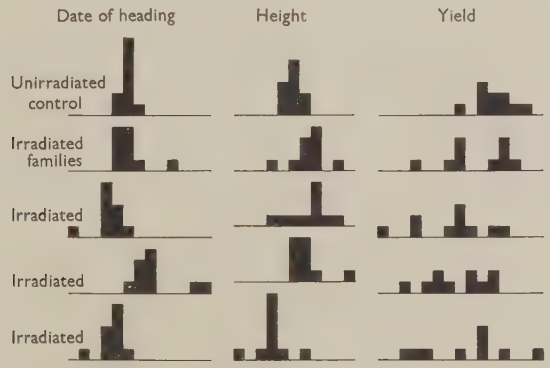

Fig. 1. Increase in genetic variability within X_4 generation families of S. 147 oats after irradiation with X-rays.

The material was examined critically throughout the growing season for evidence of variation in agronomically important characters, especially height, earliness and apparent yield. Finally thirty families were selected as showing, in one or more lines within each family, some variation from the unirradiated standard, together with another eight randomly selected families, and two check families derived from unirradiated material.

The 400 individual lines were grown in the X_4 generation in a replicated experiment. Because of limited supply of seed each plot was represented by a single drill 6 ft. long. To obtain the best information on intra-family variation the ten lines within each family were grouped together and the whole families randomized over replicates. During the season detailed measurements again were made on plant height, earliness of heading and yield, as well as observations on disease incidence and lodging. The increased variability found in some families in respect of plant height, earliness, and yield, compared with an unirradiated control family, are to be observed from Fig. 1. The top row of histograms represents the variation of one of the unirradiated control families for earliness, plant height and yield. The spread is least for heading date, and most for grain yield, as would be expected from a knowledge of the relative effects of micro-environmental factors on these characters. It is readily apparent, however, that in the remaining histograms the spread is much greater than in the control, indicating that heritable variation in these characters had been induced in the experimental material.

From the data collected in this trial twenty-three individual lines were selected for further testing in a more precise X_5 generation trial with larger plots, again replicated four times. Unirradiated S. 147 seed was used as the check variety and a selection of results is shown in Table 1.

Table 1. *Height, earliness and yield of selected X_5 generation lines of irradiated S. 147 oats compared with an unirradiated control*

Line	Height (in.)	Heading date (days after control)	Yield (control = 100 %)
Control	27·9	0	100
A	25·9	+2·5	84*
B	24·8*	−1·0	93
C	24·1*	+1·5	80*
D	26·3	−4·0*	92
E	23·6*	+1·5	87
F	27·1	+0·5	108
G	25·2	+1·0	76*

* Significant difference ($P = 0{\cdot}05$).

Differences were found in all characters studied, although significant yield variations were only in the direction of lower yield, nevertheless, certain lines did show a consistent tendency to increased yield in both X_4 and X_5.

Subsequent to the X_5 generation promising material was included in routine trials of varieties and breeding material for more extensive comparison with the best types of oat at present available. In these trials, which can be considered to be outside the irradiation investigation *per se*, some of the earlier findings were confirmed but it was also apparent that none of the mutant lines was likely to be of agricultural use because none was an improvement on the best types derived by intervarietal crossing.

The prime purpose of this investigation was to evaluate the method as a breeding technique and to assess possible disadvantages compared with other methods. One of these disadvantages lies in the fact that irradiation can be expected to produce only occasional random variants among a mass of totally unchanged material, and selection in subsequent generations must be capable of isolating these, whereas after hybridization of suitably selected parental types, the usual method of releasing variability, a continuous range will be present for selection to operate upon.

As would be expected it was found that the ease with which these isolated aberrants could be identified varied with the degree to which the character concerned was influenced by the small environmental variations that usually occur in field experiments. As a result of this the nature of the improvement aims needs to be taken into account when considering the possibility of using an irradiation technique for improvement of a crop such as a cereal. Characters involving obvious morphological variation can be identified on single plants at the X_2 generation; thus very large populations of irradiated material can be handled. In the case of the continuously variable characters at present studied the situation was found to be as follows in both S. 147 and S. 172.

(1) The maturity date variants could be readily identified at the unreplicated X_3 panicle row stage.

(2) The straw length variants could be identified with worthwhile accuracy at the same stage and confirmed with accuracy in the replicated X_4 trial.

(3) Yield variants, except grossly low-yielding lines, could not be identified with any precision whatever in X_3. It was found in fact that lines selected at this stage for apparent yielding ability were no better than the range shown by the randomly selected families. However, it was found that selection based on the X_4 results was moderately efficient in that the highest yielding lines taken at this stage maintained their superiority in X_5 although not significantly different from the control.

Thus it would appear that only in replicated trial would there be any likelihood of identifying

small improvements in yield brought about by irradiation. A trial covering an adequate amount of material to compensate for the very low expected mutation rate would be of prohibitive size. For example, for all the X_3 material of S. 147 in the present investigation to be put into an X_4 trial similar to the one grown, an area of land approximately 6 acres in extent would be required. At the commencement of an irradiation-induced improvement programme it is necessary therefore to consider carefully the aims because the greater the ease with which the desired character can be identified the larger the number of irradiated units (seeds) which can be adequately tested.

It was stated earlier that two different dosages of irradiation were given to the S. 147 material which was subsequently treated as a homogeneous whole for experimental purposes. It was found that at the X_3 generation, the last stage before the application of selection pressure, 69 % of the material had received the lower dosage. At the X_5, after two cycles of selection the proportion had risen to 78 % and of the seven significant results in the X_5 trial only one, a lower yielding selection, came from the higher dosage material. Whilst undue significance cannot be attached to these figures, they indicate that doses well below the lethal level are most useful for producing small quantitative variations.

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Induced tolerance of stored-product beetles to methyl bromide

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The response of three species of stored-product beetles to methyl bromide has been studied since 1953. The species used in this work have been *Sitophilus granarius* (L.), *Tribolium confusum* Duv., and *Tenebroides mauritanicus* (L.). A preliminary report of progress and an account of the materials and methods employed was given by Monro & Upitis (1956). Up to 1960 the only results of interest have been obtained with the first-named insect, the granary weevil, and the present report is confined mainly to this species.

In 1956 a population of the GG strain of *Sitophilus granarius*, reported by Musgrave & Miller (1958) to contain mycetomal micro-organisms, was brought into the selection programme.

So far, in all this work, the fumigant has been applied to the adults. They have been exposed under uniform environmental conditions for 5 hr. at 25° C. and 70 % r.h., the only variable being the dosages of methyl bromide required to produce the desired mortalities at each stage of selection.

ACQUIRED TOLERANCE OF POPULATIONS OF 1953

The three populations of *S. granarius* chosen originally for this study were the Montreal Wild (MW), the London (Ontario) Wild (LW), and the laboratory strain (R). It should be pointed out that, in order to give as large a gene pool as possible, the two wild populations were derived from wide collections made in the original localities. The progress of the acquisition of tolerance of the two wild strains, under pressure from methyl bromide at each succeeding generation, is shown in Fig. 1. The response of the R strain has followed a similar pattern, but the degree of tolerance reached has been less. Table 1 shows that in terms of weight of fumigant

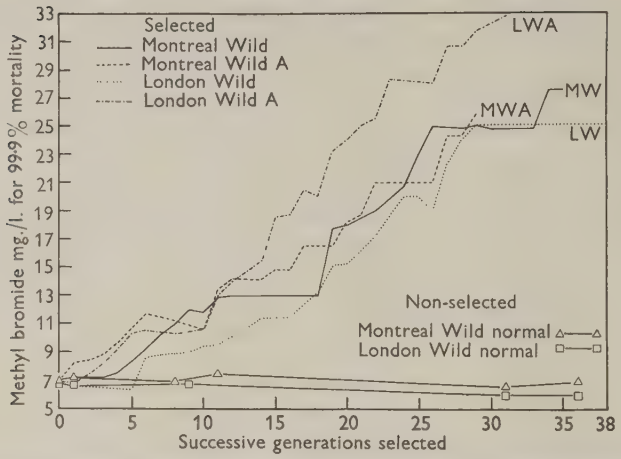

Fig. 1. History of selection of increased adult tolerance to methyl bromide fumigation of four stocks of *S. granarius* compared with normal non-selected stock.

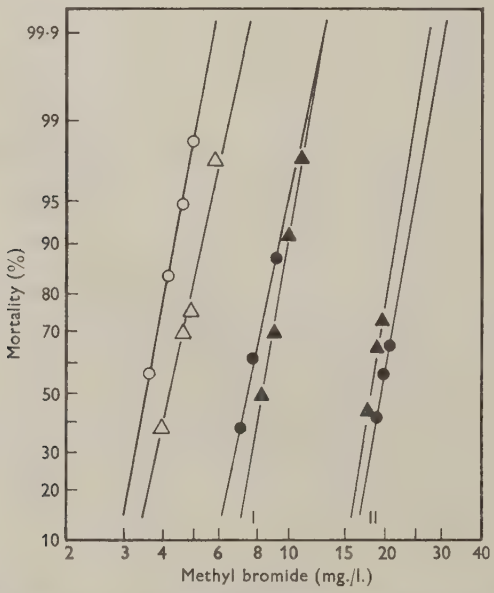

Fig. 2. Mortality lines for two stocks of *S. granarius*, selected for tolerance to methyl bromide fumigation, compared with the corresponding non-selected stocks at two stages in the programme of selection. ○, London Wild A normal strain; ●, London Wild A selected; I—gen. 11; II—gen. 29. △, Montreal Wild normal strain; ▲, Montreal Wild selected: I—gen. 11; II—gen. 34.

required for LD 50 the selected LWA strain requires 5·5 times that for the normal non-selected strain. The suffix 'A' after the letter designation of the strains indicates that these were selected subsequent to 1956 from survivors of exposures above the LD 50 mark, as opposed to the other strains which continued to be propagated from survivors from treatments above LD 75. The fact that the 'A' strains have attained a greater tolerance than the others tends to confirm the findings of King (1955), who showed with *Drosophila melanogaster* Meigen that resistance developed more rapidly if the gene pool was kept as wide as possible. During all this time the response of the non-selected populations has remained at a constant level over the 7-year period. There is as yet no indication that a maximum degree of tolerance has been reached by any of the populations. Fig. 2 shows regression lines for LW, LWA, MW and MWA for response to the fumigant at three stages during the progress of the work. During this time the regression lines have moved slowly towards the right and at no time has there been deviation from parallelism.

After the thirteenth selection a portion of the MW strain was removed from selection pressure and tested at every subsequent generation. Fig. 3 shows that there has been no indication of reversal towards the response of the normal strain after twenty-six generations of suspended selection.

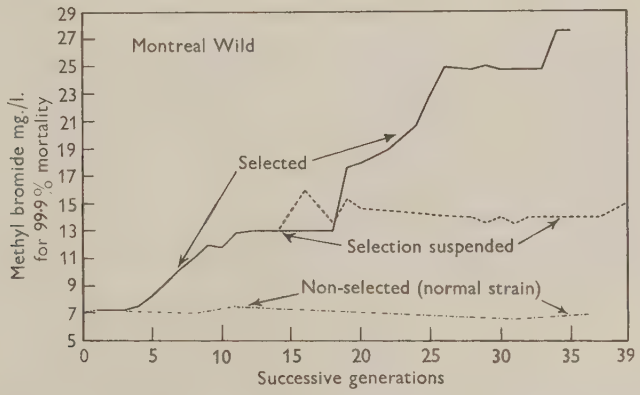

Fig. 3. Effect of suspension from selection by methyl bromide of one portion of a population of *S. granarius*, compared with continuance of selection of the remainder.

In every instance the body weight of the selected strains is significantly greater than the normal; for example, the average weight per individual (based on 100 adults per sample) has increased as follows: LW 2·872–3·560 mg., an increase of 23·9%; MW 2·418–3·512 mg. (45·2%). Although as yet no detailed study has been made of differences in the length of the life cycles of the different strains, observations made during the routine of the rearing programme have revealed no noticeable differences.

Populations of *Tribolium confusum* and *Tenebroides mauritanicus* have been subjected to a régime of selection by the fumigant for the same length of time. Some tolerance to methyl bromide has been acquired by both species, but so far it is of a low order compared with that found for *Sitophilus granarius*.

TOLERANCE TO OTHER FUMIGANTS

Now that an appreciable tolerance to methyl bromide has been acquired by the various strains of *S. granarius* their response to other representative fumigants has been tested. The results obtained with the LWA population, shown in Table 1, are typical. All the differences between the normal and selected strains at LD 50 are significant but the ratios for two of the fumigants (HCN and chloropicrin) are rather low. The 'Tolerance Ratio' is obtained by dividing the LD 50 of the selected strain by that of the normal strain.

RESPONSE OF A STRAIN WITH MICRO-ORGANISMS

Musgrave & Miller (1958) have reported the finding of micro-organisms in the mycetome of larvae of a strain of *S. granarius* now known as GG. The identity of the micro-organisms is uncertain but these two authors have suggested (1958) that they may prove to be Actinomycetes. During the metamorphosis of the insect the mycetome breaks down and the mycetocytes, of which it is composed, together with the mycetomal micro-organisms are found in the adult insects in the mesentera. They are also found in the female gonads and are passed by this route from one generation to another.

Table 1. *Response to fumigants of a strain of* Sitophilus granarius (*London Wild A at 27th selection*), *more tolerant to methyl bromide, compared with normal non-selected strain*

Dose in mg./l. (with 95 % confidence intervals) required for 50 % mortality for 5 hr. at 25° C.

Fumigant	Dose		Tolerance ratio (with 95 % confidence intervals)
	Selected	Normal	
Methyl bromide	19·7 (19·2–20·2)	3·6 (3·5–3·7)	5·5 (5·3–5·6)
HCN	16·4 (15·6–17·2)	8·2 (7·5–9·0)	2·0 (1·8–2·2)
Acrylonitrile	4·9 (4·3–5·6)	1·05 (0·96–1·15)	4·7 (4·0–5·4)
Ethylene oxide	20·1 (19·5–20·7)	4·1 (3·9–4·4)	4·8 (4·4–5·2)
Chloropicrin	6·6 (6·0–7·3)	3·9 (3·5–4·4)	1·7 (1·5–1·9)
Phosphine	13·0 (11·8–14·3)	2·2 (1·6–2·9)	5·9 (4·3–8·0)
Ethylene dibromide	8·5 (8·2–8·8)	2·85 (2·7–3·0)	3·0 (2·8–3·2)

Table 2. *Response to fumigants of a strain of* Sitophilus granarius (*GG A at 12th selection by methyl bromide*) *without mycetomal micro-organisms, compared with a normal unselected strain still containing micro-organisms*

Dose in mg./l. (with 95 % confidence intervals) required for 50 % mortality for 5 hr. at 25° C.

Fumigant	Dose		Tolerance ratio (with 95 % confidence intervals)
	Selected	Normal	
Methyl bromide	10·9 (10·7–11·1)	4·5 (4·4–4·6)	2·4 (2·2–2·6)
HCN	13·1 (12·4–13·8)	14·0 (11·4–17·2)	1·07* (0·9–1·3)
Acrylonitrile	3·2 (2·9–3·4)	1·1 (1·1–1·2)	2·8 (2·5–3·1)
Ethylene oxide	8·7 (7·9–9·6)	5·3 (5·1–5·5)	1·6 (1·5–1·8)
Chloropicrin	6·4 (5·8–7·0)	2·9 (2·5–3·3)	2·2 (1·9–2·6)
Phosphine	25·5 (21·8–29·8)	2·0 (1·5–2·7)	12·8 (9·1–17·8)
Ethylene dibromide	7·2 (6·9–7·6)	3·5 (3·3–3·6)	2·1 (1·9–2·3)

* No significant difference in tolerance ratio between selected and normal strain.

S. granarius certainly seems to occur in several strains. Zacher (1934) described a small pale form from Africa. Mansour (1935) claimed there was an Egyptian variety that was paler than normal, and which did not harbour the characteristic mycetomal micro-organisms. For some years a strain apparently similar to this has been reared in the laboratory in Canada (Musgrave & Miller, 1958). This strain was originally collected in the Port of Montreal by H.A.U.M. and was referred to as the Montreal Wild (Monro & Upitis, 1956), from which the initials MW were derived. This MW form is paler in colour and lighter in weight than the GG strain mentioned above, which almost always harbours great numbers of mycetomal micro-organisms (Musgrave & Miller, 1958).

Though efforts to isolate and cultivate the mycetomal micro-organisms from the weevils have not met with success, other micro-organisms have been isolated and identified (Crawford, McDermott & Musgrave, 1960).

In 1956 the GG strain was introduced into the methyl bromide selection programme. After twenty-three generations of selection it has been found that increased tolerance to the fumigant has developed in much the same pattern as with the other strains. Furthermore, the selected strain has become pale in colour like the MW strain. Also, a survey of the normal and selected GG strains has indicated that tolerance to methyl bromide is now associated with scarcity or absence of the mycetomal micro-organisms characteristic of the normal GG strain (Musgrave, Monro & Upitis, 1961).

These two strains of GG have now been tested for response to other well-known fumigants and the results are summarized in Table 2. When these results are compared with those given for the LWA selected strain in Table 1 it is seen that the response of the GG strain to selection by methyl bromide has produced a tolerance to this and other fumigants similar in degree to that for the MW strain, regard being paid to the shorter period of selection. However, against phosphine the tolerance developed is greater than with the LWA strain. The interesting exception is with HCN, where the LD 50 for the two strains is approximately the same.

As with all the strains selected for tolerance to methyl bromide, the selected GG weevils are heavier than the original strain. (Average increase 3·304 to 3·938 mg., 19·1 %.)

DISCUSSION

The tolerance induced in successive populations of *S. granarius* exposed to methyl bromide is shown by a shift in the dosage/mortality lines, unaccompanied by change of slope. This tolerance is retained for at least twenty-three generations after the cessation of exposure to the insecticide, and is accompanied by an increase in tolerance to other fumigants not chemically related to methyl bromide. The mean body weight of the tolerant population shows a significant increase. In general the principal characteristics of the selected populations fall within the concept of 'vigour tolerance' as originally defined by Hoskins & Gordon (1956) and discussed more recently by Brown (1958). Vigour tolerance is postulated as being the result of a non-specific improvement in the physical and biochemical condition of the insects brought about by polyfactorial inheritance, each factor having a relatively small effect. It is of interest to note that Parkin & Lloyd (1960) have also reported a weight increase in a strain of *S. granarius* exposed to the selective action of pyrethrins in oil solution.

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A study of the development of beetle infestations in flour-milling machinery

By C. E. Dyte

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Many species of beetles have been recorded from flour mills, but relatively few are able to breed within the milling machinery so as to produce a serious infestation. The beetles most commonly found breeding in this environment are the Tenebrionids *Tribolium confusum* Duv. and *Gnathocerus cornutus* (Fab.), and the Cucujid *Cryptolestes turcicus* (Grouv.). The other common pest of milling machinery is the Phyticid moth, *Anagasta kühniella* (Zell.). These insects are essentially pests of those parts of the mill concerned with flour, and are not major pests of stored grain; in fact there are usually closely related species which are more often found in grain (e.g. *T. castaneum* Hbst., *C. ferrugineus* (Steph.) and *Ephestia elutella* (Hb.)).

Flour-mill insects are normally controlled by fumigation, but this is usually restricted to that section of the mill which contains the milling machinery. Re-infestation from other sections soon occurs and most flour mills are fumigated regularly every year.

The beetle populations developing in nine centrifugals (rotary sieving machines) of a London flour mill were sampled, at fortnightly intervals over a period of 3 years, by removing all the insects and flour adhering to the insides of the canvas-covered frames which form the sides of each centrifugal. The residues on these centrifugal panels were all flours, and had passed through sieves with apertures of about 0·1 mm., which would prevent the passage of the eggs of any of the species found. The populations studied were therefore not reinforced by insects entering the machines with the moving millstocks. Sampling began in January each year, and continued until the mill was fumigated in June, July or August.

The species present in the largest numbers was *Cryptolestes turcicus*. In January, individual machines rarely yielded more than ten insects per visit, but just before fumigation the badly infested centrifugals yielded several hundred beetles, the maximum number recorded being 947 adult *Cryptolestes* from one machine in July 1959. The total numbers of *Cryptolestes* found varied from year to year, but the proportion of the total infestation in the different machines was fairly constant. Centrifugals A 9 and B 11 supported the largest infestations every year.

Larvae of *C. turcicus* and both adults and larvae of *Tribolium confusum* and *Gnathocerus cornutus* were found in relatively small numbers. However, most of the *Cryptolestes* larvae obtained each year were found in centrifugals A 9 and B 11, as were most of the adults and larvae of *G. cornutus* in the only year in which the species was present in moderate numbers.

Observations of the moisture contents of the millstocks in the nine different machines were made in 1960. The results gave a complicated picture but the numbers of adult *Cryptolestes* found in the different centrifugals in that year were positively correlated with the average moisture content of the respective stocks. The two centrifugals (A 9, B 11) which supported the heaviest infestations were those which contained the least nutritious flours as assessed by analyses for thiamine, iron, oil, protein, ash and fibre.

It appears that the moisture content of the millstocks is of importance in determining where large beetle infestations may occur, but that the nutritional value of the stocks has little significance in this respect. Such information emphasizes the importance of studying the complex of environmental factors under 'field' conditions. Moreover, it is of practical importance in the planning of local control measures such as spot-fumigations.

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The importance of plant-parasitic nematodes in Britain

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Quantitative data on the proportion of crops or land infested with various species of eelworms are very limited and as there is little information on the relationship between population density and yield it is very difficult to assess the importance of eelworms on the basis of crop losses. There have been some national and local surveys on a few species, but much of this review is based on personal experience in eastern England supplemented by information kindly supplied by colleagues in other regions.

Undoubtedly the most important species is potato-root eelworm (*Heterodera rostochiensis* Woll.) which affects potatoes and tomatoes. As a result of publicity and N.A.A.S. soil sampling to detect the higher infestations, serious failures are much less common than they were 10–15 years ago when many farmers had to reduce their acreage because of eelworm. In addition to the obvious losses there are also concealed losses because farmers may have to grow potatoes on fields less suited to the crop and which do not yield such a high return. The position has eased recently as a result of the national reduction in potato acreage.

A very high proportion of fields in all the main potato areas is infested at a detectable level and random surveys in eastern England have shown this figure to be 84 % for Holland, Lincs. and 42 % in the Isle of Ely. In both counties about 10 % of the acreage is infested at a level which will cause appreciable crop loss, although soil sampling gives information that leads to avoidance of planting in a high proportion of such fields.

On tomatoes, potato-root eelworm is also a major pest over most of the country with the exception of the Lee Valley, which has the largest acreage of glass. Stone (1951) in Somerset, and Lewis (1960) in South Wales, have carried out random surveys of tomato houses and found that 35 and 43 %, respectively, of glasshouses were infested and in both areas about 10 % showed a level of infestation likely to reduce yield. Thompson (1959) stated that 80 % of nurseries in Yorks. and Lancs. were infested.

Experience with potato-root eelworm had made advisers and administrators feel that it was worth making great efforts to prevent other species of root eelworms increasing to the same extent, and this was the reason for the introduction in 1943 of the first Beet Eelworm (*Heterodera schachtii* Schmidt) Order to control cropping in affected areas. Our records show that nearly 20,000 acres are infested, which I estimate is about 1–2 % of the beet-growing area. Similarly *H. göttingiana* Liebscher, though not important as a pest at present (Brown, 1958), is recognized as a potential threat to the rapidly expanding vining pea industry. *H. carotae*, first recorded by Jones (1950), is now known to occur in about forty fields in Eastern England and has already caused some failures.

Cereal-root eelworm (*H. major* O. Schmidt) is rather different. Although it has been known in this country for a long time it seemed to flare up in the early 1950's and surveys show that it was present in 43 % of fields through the country (Southey, 1955). On light land it can be a serious pest of oats and, to a lesser extent, of wheat. Subsequent experience seems to indicate that outside certain limited areas it is less of a menace than we originally thought. Probably this is because it is less persistent than some other root eelworms, and perhaps there are unknown seasonal factors which affect its build-up and decline.

Root-knot eelworm (*Meloidogyne* spp.) is a much more serious pest in tropical countries than it is here, where it is mainly confined to glasshouses and is most familiar on cucumbers and tomatoes. Its effects on yield are not always as devastating as one might imagine from the appearance of infested roots, and it is one of the few species where chemical control is helpful.

There are a number of distinct biologic races of stem eelworm (*Ditylenchus dipsaci* (Kühn)) and one of the best known is the race which attacks oats. Although this pest is generally widespread, it is mainly in the northern parts of the country that its effects are most serious and

McLeod, Golightly & Price (1954) showed in a random survey of 116 oat crops on thirty-seven farms in Durham that twenty-two farms and 30 % of the acreage was infested, 10 % of the fields at a level likely to affect yield. Fortunately good varieties of oats are now available which are resistant to this race. Probably the race of stem eelworm most important economically is that affecting narcissus and daffodils. In the 1920's this was causing havoc in our bulb stocks, but the introduction of warm-water treatment in the early 30's brought it gradually under control, and nowadays most bulb growers treat their stocks at least every other year, the cost of this being about 3 % of the annual value of the crop.

The tulip race of this eelworm has only come into prominence in the last decade, and a recent survey (George & Southey, 1956) showed it to be present in about 11 % of the stocks in Holland, Lincs., where most of our tulips are grown. This situation is perhaps similar to that in narcissus 40 or 50 years ago, but at present it has not been possible to devise a warm-water treatment for tulips satisfactory for all varieties and seasons.

Chrysanthemum eelworm (*Aphelenchoides ritzema-bosi* Steiner) can feed ectoparasitically, causing distortion and blindness of cuttings as well as the better-known endoparasitic attack in which the leaves are gradually killed from the base of the plant upwards. The eelworm is certainly a major problem for most chrysanthemum growers, but parathion treatments of stools and cuttings have now largely replaced the tedious and expensive warm-water treatment. In eastern England the majority of the larger growers use parathion and the cost is very small, representing only about £10–15 out of a crop value of about £5000 per acre. Nevertheless, it is quite clear from occasional mistakes and omissions of treatment that the eelworm could rapidly cause serious losses if unchecked.

I think that the species I have mentioned are the most important in the country but I might also include the lucerne race (Brown, 1957) and the red clover race of *D. dipsaci*. Although Lester & Large (1958) showed that the red clover race was widespread, serious attacks only occur locally. *A. ritzema-bosi* and various races of *D. dipsaci* are occasionally serious and often a nuisance on strawberries. Other species of nematodes occur on various crops but are not sufficiently serious and widespread to justify special mention here.

I have not referred to eelworms as vectors of disease nor the extent to which their occurrence interferes with trade both nationally and internationally and in looking at the national picture we must not forget the serious losses which may be caused to the individual.

In reviewing the present position I cannot help feeling that the potential threat of species now relatively uncommon demands as much, if not more, attention than some of the familiar species which we are learning to live with. This threat can only be dispelled by increased research and its practical application.

I am indebted to many N.A.A.S. colleagues for information included in this paper.

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Practical problems and recent trends in nematode control

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I propose to limit the scope of this paper to recent, and possibly future, trends in chemical control of nematodes on the crop, as distinct from plant quarantine measures such as fumigation of seeds, dipping of bulbs and tubers.

Many of the nematicides currently in use have been giving good service for 10 years or more—materials such as chloropicrin, methyl bromide, dichloro-propene dichloropropane, and ethylene dibromide. These are all *halogenated hydrocarbons*, materials toxic in themselves to nematodes and all relying on their volatile nature for dispersion in the soil. They are generally phytotoxic and are applied to fallow soil, toxic residues being dispersed by cultivation and aeration of the soil before sowing or planting the crop. This practical limitation has led to a search for more active and less phytotoxic compounds, the most notable of which is the comparatively recently introduced dibromochloropropane—a compound some ten times as active as D.D. mixture, which allows it to be used at nematicidal dosages on certain growing crops.

In addition to this well-known family, however, several newer groups of nematicides have appeared. First, we have the *organic phosphates*. Parathion (diethyl *p*-nitrophenyl phosphorothioate) and Systox or demeton (isomeric mixture of diethyl (ethylthio) ethyl phosphorothioate) have been used for many years against foliar nematodes, whilst parathion has been used as a soil drench against root-knot eelworms under glass. A number of closely related compounds, Thimet, Disyston, Diazinon, have been used with success against, for example, *Ditylenchus dipsaci* and *Pratylenchus* spp. on bulbs. Recent developments in this group are dichlorophenyl diethyl phosphorothioate and the as yet experimental diethyl pyrazinyl phosphorothioate. These compounds are non-volatile and have low water solubility. Their advantages are high nematicidal activity and residual value, with low phytotoxicity. The main disadvantage is high mammalian toxicity.

The next group comprises the *dithiocarbamates*. The methyl ester of dimethyl dithiocarbamate was first shown to have nematicidal properties in 1941. The water-soluble sodium salt of N-methyl dithiocarbamic acid, introduced in 1955, has proved a useful tool in soil sterilization since it possesses also high fungitoxicity. The drawback to these compounds is their phytotoxicity. Nematicidal action is reported to be due to breakdown in the soil to give methyl isothiocyanate.

Various *sulphur-containing compounds*. First, dimethyltetrahydrothiadiazine thione, which is believed to break down in soil to give first the dithiocarbamate and then methyl isothiocyanate, and chlorophenyl methyl rhodanine which also is believed to yield methyl isothiocyanate in soil. Both are therefore limited in use by phytotoxic risk, but of course in certain circumstances where weed control is important as, for example, tobacco seed-beds this may become an advantage. A group of compounds of low phytotoxicity are the tetrahydrothiophene dioxides, the more active of which are halogen substituted.

And finally, *mercury compounds*. It is rather surprising that so little has been done with this group, since mercuric chloride has been used for years as a standard in nematicide screening tests. However, recent work in Scotland has shown encouraging results with yellow oxide of mercury when applied as a solid with a machine designed to give a high degree of admixture with the soil and to cut out the risk to the operator of working with a highly toxic dust.

In addition to these compounds, there has been in recent years a bumper crop of newly patented materials, about which insufficient data exist for discussion at the present time. Most of these fall into one or other of the groups I have just reviewed, with a preponderance of organic sulphur compounds—thiocarbamates and complex isothiocyanates. In a different category is phenylazoaniline, reported to be safe to use and non-phytotoxic.

So much for the development of nematicides over the past 10 or so years. What are the

current trends in nematicide application and thought? I think one of the most important recent changes in attitude has been the abandonment of the idea of a universally applicable nematicide and the realization that there are many different nematode problems to be tackled in different ways. For example, the problem of mixing a chemical into the soil is vastly different for the citrus grower who needs a treatment effective down to 2–3 ft., and may be in a position to irrigate his land to achieve this, from that of a cotton grower who may get adequate protection from a granular compound applied with the seed to a depth of a few inches only. The effectiveness of any chemical control measure is influenced by so many factors, cultural, climatic and biological: a material which behaves perfectly in a warm sandy loam may be useless in a silt or peat soil, or a soil which is cold or waterlogged. The question of formulation is, in itself, a reflexion of the coming-of-age of nematicides as accepted aids to better farming: in common with insecticides and fungicides, nematicides are now offered as emulsions, wettable powders and granules in addition to the straight chemical or mixtures of two or more chemicals. Another attitude which has changed in recent years has been the weakening of the call for a once-for-all 100% control and the acceptance of a measure of control sufficient to give an economic crop increase, particularly where the application can be made to the growing crop without harmful effects.

A revival of an earlier approach to chemical control has been made in the study of mechanisms of resistance in plants and in attempts to isolate naturally occurring resistance factors. It is interesting to recall that as long ago as 1925 work in this country showed that exudates of several spp. of mustard were nematode-inhibiting. A recent example of this lead is the American work with extracts of asparagus root. Asparagus is apparently naturally resistant to the eelworm *Trichodorus christiei* and a glycoside (as yet unidentified) isolated from asparagus roots is claimed to reduce *Trichodorus* numbers when applied to the soil as a drench or when sprayed on the leaves. Another example is that of *Tagetes erecta*, the African marigold, a plant naturally resistant to several nematode genera, but particularly to species of *Pratylenchus*. Here the root exudate has been shown to be toxic to nematodes in the soil, and a nematicidal principle has been isolated from it and identified as the compound terthionyl, or tri-thiophene; it is interesting to note the relationship with the thiophene dioxides previously mentioned. Many species of *Crotalaria*, a legume commonly grown in tropical countries for green-manuring, have been shown to reduce root-knot eelworm populations. It may be that there is a similarly extractable nematicidal compound in this genus.

Systemic nematicides, until recently, have been regarded as being useful only in the control of foliar and stem-and-bulb eelworms. Selenium compounds were employed many years ago as a soil application against chrysanthemum eelworm and several of the organo-phosphorus compounds have more recently been shown to be effective against stem-and-bulb eelworms when sprayed on the foliage.

It is claimed that asparagus root extract is toxic to *Trichodorus christiei*, a root-feeding nematode, when sprayed on the foliage. This novel approach, control by chemically induced resistance, stems from work on the selective weed-killers many of which are rapidly translocated downwards from foliar application and have been shown by radioautographs to move into the root.

In 1954, work in America showed that a growth-regulatory chemical, α-methoxy-phenyl acetic acid, applied to the leaves of one plant was exuded from the roots in sufficient quantity to produce symptoms in an adjacent plant, and the same workers have since shown that mandelic acid and certain trichloro- and tetrachloro-benzoic acids exhibit the same properties. It only needed the report, in 1958, that maleic hydrazide sprayed on tobacco inhibited the development of root-knot eelworm to show that here was a possible means of control which could cut out the difficulties and phytotoxic hazards of soil application.

Let us look at the ways in which, theoretically, a foliar-applied chemical could interfere with the normal host–parasite relationship. We have heard how eelworms are attracted to plant roots. Whether one believes that they are attracted to an environment determined by the actively respiring root tip, or to a chemically definable substance exuded from the root tip, it will be seen that in one way or another a translocatable substance may be able to render the root unattractive, i.e. by interfering with respiration, inhibiting the formation of exudate or changing the composition of the exudate. Secondly, the translocatable substance may, on diffusion from the

root, be actively repellent, or, thirdly, it may be toxic. Fourthly, if the substance is physiologically active in the plant, eelworms may not be prevented from entering the root, but the normal plant response—formation of the giant cell complex—may be inhibited and the eelworm fail to develop; and fifthly, the substance or a metabolite may accumulate in the root tissues in toxic concentration and kill eelworms that have gained entry, or prevent their development.

In experiments at Jealott's Hill it has been found that a large number of chemicals fulfil the requirements of being mobile in the plant and exerting an inhibitory effect on the eelworm, once these materials are in the translocation stream. By this I mean that many chemicals when fed into the stem of a plant using a woollen wick have given the effect we are looking for. The snag is that this number is drastically reduced by inability to penetrate the leaf surface, or by phytotoxicity on penetration, when they are sprayed on the foliage.

Maleic hydrazide, sodium fluoroacetate and fluoroacetamide were applied as foliar sprays 2 days before inoculating the soil with root-knot eelworm larvae; the soil was covered during spraying and afterwards until the foliage was dry. With these compounds the level of phytotoxicity was still too high at rates which produced an almost clean root, although with sodium fluoroacetate and fluoroacetamide the effect was a transient foliar scorch only. Phytotoxicity apart, an appreciable degree of protection from nematode attack was conferred on the plants. So far, however, it has only been possible to do this under experimental conditions which offer a much less severe test than would the normal environment. In our experiments uninfested plants are sprayed and some days later these are inoculated with 600–700 root-knot larvae per pot. This gives the chemical time to reach the root before the eelworm attack develops. We have not yet been able to control nematodes already established in the root tissues.

Mode-of-action studies lead us to believe that maleic hydrazide represents the first group which I spoke about earlier, that is, compounds which interfere with the attractiveness of the root tip; it does this, we think, by inhibiting production of the attractive root exudate. Sodium fluoroacetate and fluoroacetamide on the other hand are highly nematicidal *in vitro* and it seems likely that they belong either to the third group, toxic compounds which are exuded from the root, or to the fifth group—those which accumulate in toxic concentration in the root tissues. With all these compounds it has been shown that nematodes which are established in the root before foliar treatment are apparently unharmed by treatment, both parasite and gall developing normally.

Is this due to insufficient mobility of the chemical resulting in a sublethal concentration in the root tissues? Or does the giant-cell complex protect the parasite from toxic materials in the translocation stream? If this obstacle were overcome would the effect of such a systemic nematicide persist long enough to be worth while? These are points which must be answered before the practicability of this means of control can be demonstrated. American work with a large number of chemicals trunk-injected or implanted in spreading-decline-affected citrus trees failed to show any improvement due to treatment.

To return for a moment to maleic hydrazide, I said that we believed that the main effect of maleic hydrazide was to render the roots unattractive by stopping growth, and hence by inhibiting the production of attractive diffusate. The fact that root diffusate production *is* inhibited may be demonstrated by cyst-hatching tests with potato-root eelworm using leachates from sprayed and unsprayed plants; and in similar experiments with other test compounds there have been indications that the cyst-hatching capacity of treated plants can be markedly decreased by certain chemicals and increased by others. May I suggest that experiments on these lines, whilst unlikely to lead directly to a novel means of control, might at least throw some light on a possible relationship between the hatching and attracting properties of root exudates?

In conclusion, perhaps I should return to the more practical aspect of nematode control. I hope I have shown that there are, and will be increasingly in the future, different kinds of nematicide for differing nematode problems. I would like to stress the importance of using these materials as supplements to good farming practice, in the light of the fullest possible biological knowledge of the many factors involved.

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Viruses isolated from cherry trees with rasp-leaf and leaf-roll diseases

By R. Cropley

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(*Abstract*)

Rasp-leaf and leaf-roll diseases of sweet cherry are widespread in England, and in some areas their prevalence causes orchards to become commercially unprofitable.

The leaves of rasp-leaf trees are reduced in size, with enations on their undersurfaces. Transmissions to herbaceous hosts by sap inoculation and serological tests with the isolated viruses indicate that raspberry ringspot virus is the cause of rasp leaf in most orchards, but that arabis mosaic virus can cause a similar disease.

Cherry leaf-roll virus, which is causing the death of cherry trees in several Kentish orchards, is readily transmissible by sap inoculation to a wide range of herbaceous hosts. The herbaceous host reactions, thermal inactivation point and particle size and shape are characteristic of a soil-borne 'ringspot' virus. Plant protection and serological tests indicate that this virus is distinct from arabis mosaic, raspberry ringspot, tomato black-ring, lettuce ringspot, tobacco ringspot and tomato ringspot viruses. Cherry leaf-roll virus is sap-transmissible from tobacco to cherry and strawberry, and from cherry to *Prunus pennsylvanica*. The virus can be identified serologically in cherry sap extracted from expanding buds or young leaves in the spring, but not during the summer and autumn months.

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Report of the Council of the Association of Applied Biologists for the year 1960

The Officers and other Members of the Council of the Association were as follows:

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Forty-two new Ordinary Members were elected during 1960, and four resignations were accepted during the year. Membership was 770 on 31 December 1960, 165 being resident overseas, but 9 Members resigned at the end of the year. Six others ceased to be Members in accordance with Law VII. One Member was elected as from 1 January 1961 on which day the Membership was 755 (718 Ordinary Members, 15 Honorary and 22 Life Members), an increase of 20 over the previous year.

Honorary Membership of the Association was conferred on Kenneth M. Smith, C.B.E., D.Sc., Ph.D., F.R.S., formerly Director of the A.R.C. Virus Research Unit, Cambridge, distinguished for his work on plant and insect virus diseases; he joined the Association in 1920; and on Prof. Dr Johana Westerdijk, formerly Director of the Centraalbureau voor Schimmelcultures, the Netherlands, and Professor of Plant Pathology at the Universities of Utrecht and Amsterdam, distinguished for her services to mycology and plant pathology.

The Council were grieved to hear of the deaths of the following Members: Dr H. F. Barnes on 5 February 1960 (see Obituary in *Ann. appl. Biol.* **48**, 205); Dr T. P. McIntosh on 26 March 1960; Mr D. Wheatley; Dr M. Wilson in July 1960; and Dr C. C. V. Batts on 18 December 1960.

Dr C. C. V. Batts died at the early age of 38. After taking an Honours Degree in Agricultural Botany at Reading in 1945 he joined the Edinburgh and East of Scotland College of Agriculture as an Assistant in Agricultural Botany, and became Plant Pathologist in the same Department in 1948. While at Edinburgh his rust studies were stimulated by Dr Malcolm Wilson and remained his greatest interest. He left Scotland for the N.I.A.B., Cambridge in January 1958, to start a new department for the assessment of disease resistance in variety trials. The excellent work done by him in this field has been summarized in a series of papers in the *Trans. Brit. mycol. Soc.* and the *Ann. appl. Biol.*

In September 1956 he joined the Department of Botany, Imperial College, to start a new one-year postgraduate course in plant pathology. He tackled this job with his usual energy and enthusiasm, and the success of the venture may be gauged from the fact that in his last term at Imperial College eight students from six countries were attending this course. He also remained active in research and supervised the work of a group of students studying various diseases of grasses and cereals. He was active in several international organizations interested in rust diseases and was a member of Council of this Association. He had been a member of Council of the British Mycological Society and Secretary of its Plant Pathology Committee.

Those who knew his enthusiasm for his work will not be surprised at the range of his outside interests. He was a fine singer and had been a prominent member of several choral societies. Recently he became an enthusiastic member of the Special Constabulary.

All those in the Association who knew him, and many elsewhere, will miss him greatly, and remember him with affection. Our deepest sympathies go to his wife and the four children.

Dr T. P. McIntosh was born on 19 August 1892. After a short career in the Potash Syndicate he served throughout the First World War. He joined the Department of Agriculture for Scotland in 1919 and established an international reputation for his knowledge of the potato, which with his organizing ability, contributed so largely to the improvement of Scottish certified seed potatoes. After his appointment, in September 1945, as Director of the Department's Scientific Services, he initiated and supervised the production of virus-tested stocks of potatoes which, percolating through the 'foundation seed 'and 'stock seed' grades, has raised the health standard of Scottish seed potatoes still higher. He also initiated and took part in the scientific work which led to seed certification schemes for oats and barley. The second and third editions of his monograph on potatoes was written in association with Findlay and Whitehead as *The Potato in Health and Disease*. Although primarily an agriculturist and agricultural botanist he was also interested in a wide range of other scientific subjects.

Dr Malcolm Wilson, who joined the Association in 1914, was one of the old school of biologists. Trained under Prof. Farmer at Imperial College and later his assistant, Dr Wilson came into contact with great biologists of the time including Sir Thomas Huxley and Prof. F. O. Bower. Breadth of interest was a great feature of Dr Wilson's teaching in the Department of Mycology and Bacteriology in Edinburgh University where he was Reader from 1923 until his retirement in 1951.

To his mycological colleagues Dr Wilson was well known for his work on the Uredinales and on forest tree diseases, but fewer knew that he had an expert knowledge of the fungal rots of timber, especially of dry rot and its eradication from buildings. In the course of this work he studied, with special reference to their hatching dates, the beetles so often associated with rotting wood.

The Council noted with pleasure the Knighthood conferred on Sir Gerard Thornton, the C.B.E. conferred on Dr F. R. Tubbs and O.B.E. on Dr Mary D. Glynne, and the election to Fellowship of the Royal Society of Prof. R. L. Wain.

The President, on behalf of the Council and the Association, presented a congratulatory address to the Royal Society on the occasion of its tercentenary.

The Council met five times during 1960. Among matters discussed by the Council was the editorial policy of the *Annals*, in view of the rising costs of publication and the increasing number of papers received. The Council are very grateful for the grant of £650 made by the Royal Society to cover the deficit on the *Annals*. Because of this deficit a Special General Meeting was held on 14 October 1960 and Law V was amended to read:

'The annual subscription is due on 1 January each year. Ordinary Members shall pay an annual subscription of £2. Any Member may compound for his subscription by payment of a sum equal to twenty annual subscriptions. Any Member, after twenty-five years' membership may, at or after the age of 60, pay an annual subscription of £1 or may compound for all future subscriptions by a payment of £7.'

The subscription of non-members to the *Annals* was increased to £5. The *Annals* would remain at its present size for the time being. Council also discussed a memorial to Dr H. F. Barnes, and decided to raise a fund with the object of inaugurating memorial lectures.

The Association was represented on the Parliamentary and Scientific Committee by the General Secretary, Biological Council by Mr M. Way, National Committee for Biology (Royal Society) by the General and Programme Secretaries, British Weed Control Council by Dr E. K. Woodford and the British Standards Institution Technical Committee for Nomenclature for Pesticides by Dr J. R. Busvine and from October by Dr A. H. McIntosh.

The Council thanks Dr Busvine for his services on this Committee over several years, and the Governing Body of Imperial College, and the Trustees of the British Museum for their kindness in providing lecture theatres for General Meetings of the Association; also the Agricultural Research Council for kindly providing meeting rooms for the Council.

Six paper-reading meetings were held in London, and attendances at them justified the

decision to hold more meetings to cover the increasing activity in several fields of applied biology. There was an average attendance of 58 Members and 47 visitors. Papers read at these meetings were as follows:

8–9 *January*. Joint meeting with the British Mycological Society on 'The nature and exploitation of crop plants' resistance to disease'. G. D. H. Bell: General introduction. R. C. F. Macer: Developments in breeding disease-resistant cereals. J. C. Haigh: Disease resistance in vegetable crops. G. Cockerham: Potato breeding for resistance to viruses. R. L. Knight, Miss E. Keep and J. B. Briggs: Breeding for resistance to *Amphorophora rubi*, vector of several raspberry viruses. D. S. Kirkham and A. E. Flood: Phenolic host metabolites as factors in resistance to apple and pear scab. E. W. Buxton: The role of root exudates in pea wilt resistance. Discussion opened by S. C. Harland.

D. Lewis: General introduction. A. C. Hastie: Variation in *Verticillium albo-atrum* from hop. R. D. Tinline: Pathogenic and cultural variation in *Cochliobolus sativus*. Marion A. Watson: Interaction, or genetic recombination, between potato viruses *Y* and *C*. D. A. Doling: Physiologic race surveys. C. C. V. Batts: Physiologic specialization in cereal rusts and smuts. W. Black: Races of *Phytophthora infestans* and resistance problems in potatoes. Discussion opened by C. T. Ingold.

12 *February*. Symposium on 'Aspects of crop physiology'. Chairman: F. G. Gregory. D. J. Watson: An attempt to increase yield by controlling leaf area index. E. G. Brittain: The efficiency of the utilization of solar energy by higher plants. J. P. Cooper and K. E. Edwards: The significance of leaf area in selecting for yield in ryegrass. F. L. Milthorpe, M. M. Borah, D. W. R. Headford and E. M. Sadler: Problems concerned in the growth and tuber yield of potatoes. D. L. Abbott: The bourse shoot as a factor in the growth of the apple fruit. H. W. Barlow and A. P. Preston: An experiment on fruit thinning. A. S. Cooper: Growth trends in the tomato plant. I. Williams: Growth and development of the hop and seasonal changes in food reserves.

11 *March*. Joint meeting with the British Ecological Society on 'Ecological effects of crop production'. P. W. Richards: Natural and man made ecosystems. C. H. Gimingham: The monoculture of heather and its ecological effects on hill grazings. T. E. Williams: Effects of grass crop production on soil fertility. J. M. Hirst: Climate, ecoclimate and the outbreak of potato blight. T. R. E. Southwood and W. F. Jepson: The frit fly—a denizen of grasslands and a pest of oats. C. Muir: The influence of modified orchard spray schedules on the natural control of the fruit tree red spider mite *Metatetranychus ulmi* Koch. G. W. Heath: Agricultural practice and soil fauna.

8 *April*. Annual General Meeting, followed by Dr R. V. Harris's Presidential Address entitled 'The role of relation in applied biology'.

14 *October*. Symposium on 'Soil fauna in relation to soil formation and fertility'. G. V. Jacks: Introductory survey. D. A. Osmond and P. Bullock: Soil fauna in relation to pedology. F. Raw: Leaf burial by earthworms in apple orchards. J. Hobart: Distribution of soil mites in relation to conifer species. A. J. Hayes: Preliminary studies on the decomposition of coniferous litter. K. Bocock: Soil fauna and chemical changes in leaf litter. A. Macfadyen: The metabolism of soil invertebrates in relation to soil fertility.

11 *November*. Symposium on 'Applications of radiation in applied biology'. F. J. Ley: Radiation preservation of food. H. J. M. Bowen: Effects of ionizing radiation of interest in agriculture. D. R. Davies: The genetic effects of radiation in relation to crop improvement. T. D. Johnston: Studies on the use of radiation in oat breeding. P. B. Cromwell: Radiation disinfestation of stored products. J. D. Bletchly: The effect of γ-radiation on some wood-boring insects. C. C. McCready: Tracer methods for estimating the movement of pesticidal chemicals in plants. H. K. Porter: The use of ^{14}C in studies of plant metabolism. P. Newbould: Tracer techniques in the study of availability of plant nutrients. General discussion introduced by R. Scott Russell.

9 *December*. M. V. CARTER: The airborne phase of *Mycosphaerella pinodes*. Miss O. M. STONE: Alternate host plants of cucumber powdery mildew. A. H. M. KIRBY and E. L. FRICK: Glasshouse studies on the effects of chemicals on the incidence of powdery mildews. H. A. U. MONRO and A. J. MUSGRAVE: The response of stored-product beetles to selection by methyl bromide. C. E. DYTE: A study of the development of beetle infestations in flour milling machinery. D. S. PAPWORTH: The protection of stored cereal products by malathion admixture techniques.

27 *May*. Spring meeting at Reading. Forty-five Members and guests visited the N.A.A.S. South-Eastern Region Headquarters at Coley Park during the morning, inspecting the laboratories, glasshouses and experimental plots. Council are very grateful to the Director and specialist Officers who discussed their work with the party. After lunch at the National Institute for Research in Dairying the party divided, one group being shown round the Institute and the Development Commission Unit of Marine Biology at Shinfield, the other round the research laboratories of the Department of Horticulture of the University of Reading.

Council wish to thank Prof. O. V. S. Heath, the Director of the N.I.R.D. and their staffs for making the tours and discussions so interesting, and Prof. A. H. Bunting for making all the arrangements.

22–23 *September*. Provincial meeting at the University of Nottingham. Twenty-nine Members and thirty-seven visitors attended the morning session at Sutton Bonington on the 22nd. After the Association had been welcomed by the Dean of the Faculty of Agriculture and Horticulture, Prof. F. L. Milthorpe, the following papers were read: R. N. SMITH: A study of root surface fungi, using excised tomato roots. B. G. LEWIS: Ecology of certain pathogenic micro-organisms on potato tuber surfaces. G. E. MATHISON: The decomposition of keratin by fungi isolated from soil. B. N. K. DAVIS: Study of soil micro-arthropods and their relation to land reclamation after open-cast ironstone mining.

After lunch the party toured the Experimental Farm, the Horticulture Department and the Agricultural Sciences Department under the guidance of Professors Ivins, Hudson and Milthorpe. During the evening Prof. Chesters conducted a tour of the Botany Department in Nottingham, after which the Vice-Chancellor welcomed Members to a University Reception and later joined them for dinner. Later Prof. Addison took the party round the new Chemistry Department.

On 23 September the following papers were read: C. A. COLLINGWOOD and Miss A. M. BROCK: Aspects of blackcurrant gall mite infestations. R. GAIR and C. T. GUILE: Life history and bionomics of the vetch leaf gall midge. R. O. SHARPLES: Use of fungicides to control the sporulation of *Gloeosporium* on apple trees. W. J. WHITTINGTON and J. HILL: Growth studies on natural hybrids between perennial rye grass and meadow fescue. C. G. C. CHESTERS and A. E. APINIS: Effects of seaweed meals upon the growth of certain plants.

After lunch, 20 Members visited the Lenton Experimental Station (Messrs Boots Pure Drug Co. Ltd) and spent a very interesting afternoon in the different departments under the guidance of Mr Higgons and other members of the staff.

The Council are very grateful to the University authorities and Messrs Boots for permitting these visits, and to the several staffs concerned in their organization, especially Dr T. H. Nicholson who was the Local Secretary. It was largely due to his efforts that the meetings were so enjoyable and successful.

Report of the Honorary Editor for 1960

The demand for space in the *Annals* continued to increase: volume **48** contained 856 pages, compared with 811 in 1959, and 83 papers compared with 79: the number of plates remained unchanged at 16.

A broad classification of the subjects of the papers in volume **48**—with Proceedings papers in parentheses—gives: Entomology and insect pests, 15 (1); Mycology and fungus diseases, 16 (1); Bacterial diseases, 5; Viruses and virus diseases, 11; Insecticides and fungicides, 8 (1); Applied plant physiology, 10 (1); General applied zoology, 8 (5); General applied botany, 3 (1); Nematology, 4; Others, 3.

With the aim of reducing printing costs, the Cambridge University Press made proposals for ensuring greater uniformity between journals in the setting of article headings, summaries and reference lines. These proposals were circulated to members of the Publications Committee and generally accepted. The changes will come into use in volume **49**.

Delays in publication resulting from the printing stoppage continued into the first half of 1960. The dates of issue of the four numbers were, respectively, 6 May, 22 July, 4 October and 7 January.

The help received from members of the Publications Committee and other referees is gratefully acknowledged.

Report of the Honorary Treasurer for the year ending 31 December 1960

Income from the sale of the current volume (Vol. **48**) of the *Annals of Applied Biology* was £3496, an increase of £133 on sales of Vol. **47** during 1959. Income from back volumes and parts was £269, a decrease of £78 on corresponding sales in 1959. Income from reprints and advertisements was £354, an increase of £97 on corresponding income for 1959. Volume **48** cost £6166 to produce with a net cost to the Association of £2041, as compared with £1820 for Vol. **47** in 1959.

During 1960, £1230 was received in current subscriptions and arrears, an increase of £25 over corresponding items for 1959. The Association received a grant of £650 through the Royal Society from the Government Scientific Publications Fund. This grant is most gratefully acknowledged.

There was a deficit of £414 on the year's working as compared with a deficit of £656 in 1959.

The net assets of the Association at the end of 1960 were £4118 as compared with £4532 on 31 December 1959. All the available surplus funds are invested in National Savings Certificates, 5% Defence Bonds, and 4½% Defence Bonds and on deposit in the Westminster Bank. The estimated value of the stock of the *Annals of Applied Biology* is £264.

As the publishing account for the *Annals of Applied Biology* was received too late to permit the statement of accounts to be audited in full, the above report and statement of accounts is presented for approval subject to audit.*

*Auditor's certificate received 19 April 1961. See overleaf.

THE ASSOCIATION OF APPLIED BIOLOGISTS

Dr. *ANNALS OF APPLIED BIOLOGY*, INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1960 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To Estimated value of Stock, 1 January 1960		258 0 0	By Sales—Current volume	3495 16 3	
To Cambridge University Press:			Back volumes, parts and sets	268 19 11	
Paper and Printing	4138 8 10		Reprints and Advertisements	353 18 9	
Binding	375 16 1				4118 14 11
Carriage	303 0 11		By Estimated value of Stock—31 December 1960		264 5 0
Insurance and Sundries	117 9 9		By Balance carried down		2040 13 10
Commissions	1230 18 2				
		6165 13 9			
		£6423 13 9			£6423 13 9

Dr. GENERAL INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31 DECEMBER 1960 *Cr.*

Expenditure	£ s. d.	£ s. d.	Income	£ s. d.	£ s. d.
To *Annals of Applied Biology*, balance brought down		2040 13 10	By Members' Subscriptions		1230 1 9
To Printing and Stationery	184 2 2		By *List of Common Names*		10 2
To Postages and cheque stamps	74 16 4		By Royal Society Grant		650 0 0
To Subscriptions and Donations:			By Interest received and receivable:		
Parliamentary and Scientific Committee	7 7 0		5% Defence Bonds	100 0 0	
Biological Council	1 15 0		4½% Defence Bonds	45 0 0	
Barnes honorarium	105 0 0		National Savings Certificates	84 9 7	
To Sundry out-of-pocket expenses of Hon. Officers	22 0 0		Post Office Savings Bank	2 5 6	
To Institute of Biology—Secretarial Fee	25 0 0		Westminster Bank—Deposit Account	9 9 11	
To Refreshments and Hire of Rooms	59 6 7				241 5 0
To Audit Fee	15 15 0		By Balance being excess of Expenditure over Income for the year		413 19 0
		495 2 1			
		£2535 15 11			£2535 15 11

Dr. BALANCE SHEET AT 31 DECEMBER 1960 *Cr.*

Liabilities and Surplus	£ s. d.	£ s. d.	Assets	£ s. d.	£ s. d.
Sundry Creditors and accrued charges		2302 13 10	Balances at Bank:		
Subscriptions paid in advance		25 10 11	Westminster Bank: Current Account	127 13 5	
Life Membership Fund Reserve		379 0 0	Deposit Account	709 0 11	
Overseas Speakers' Fund:					836 14 4
As at 1 January 1960	25 0 0		2600 National Savings Certificates (Cost £1715 1s. 0d.)		2749 5 5
Expended during year			5% Defence Bonds		2000 0 0
		25 0 0	4½% Defence Bonds		1000 0 0
Excess of Assets over Liabilities:			Stock of *Annals of Applied Biology* as estimated		264 5 0
As at 1 January 1960	4531 19 0				
Less Excess of Expenditure over Income for the year 1960	413 19 0				
		4118 0 0			
		£6850 4 9			£6850 4 9

F. RAW, *Hon. Treasurer*

I certify that the foregoing accounts are properly drawn up in accordance with the books, vouchers and documents produced to me, and in my opinion they exhibit a true and correct view of the state of the Association's affairs.

19 April 1961 (Signed) J. B. BENNETT *Chartered Accountant*

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The annual subscription to the Association, which includes a copy of the *Annals*, is £2, becomes due on 1 Jan. of each year and should be paid to the Treasurer.

The price of the *Annals* to non-members of the Association is £5 per volume or $17.50 in U.S.A. Such subscriptions (payable in advance) and all communications respecting the publication should be sent to the Cambridge University Press, 200 Euston Road, London, N.W. 1, or in U.S.A. to the American Branch of the Press at 32 East 57th Street, New York, 22. Single numbers 30*s.* or $5.00.

Claims for missing numbers should be made within the month following that of regular publication.

Quotations for available back volumes may be obtained on application to the Cambridge University Press.

Notice to Contributors

Manuscripts for Publication

Contributors should address all material to Mr R. W. Marsh, Research Station, Long Ashton, Bristol. Manuscripts submitted for publication must be typewritten, double-spaced with ample margins, on one side only of white opaque paper approximately 13 × 8 in. It is understood that they are not offered to any other Journal for prior or simultaneous publication. Preference will be given to Members' papers: papers from non-members must be communicated by a Member for consideration by the Editors.

Owing to the demand for space and the high cost of printing, authors must drastically condense their manuscripts, in the general interest of all contributors. Numerical results should be presented either as tables or as diagrams, but not both; only such tables and illustrations as are essential can be published. Papers must start with a summary and conform to the usages of the *Annals* in all typographical matters. Contributors will be responsible for any excess over the usual charges allowed for corrections. Papers over 8000 words can be published only in exceptional circumstances. Quantitative biological data too extensive for complete publication should be deposited with the Librarian, British Museum (Natural History), Cromwell Road, London, S.W. 7.

References

References must be listed at the end of the article according to the 'Harvard System' as follows: name and initial(s) of author; year of publication in brackets, further distinguished by the addition of small letters *a*, *b*, *c* to the date where more than one paper published by the same author(s) in the same year is cited; exact title of paper; contracted title of periodical as given in *World List of Scientific Periodicals*; volume number in arabic figures; beginning page number of article. In the text references should be denoted by giving the name of the author(s) with the date of publication in brackets, e.g. (Brown, 1937), (Brown, 1937*a*; Jones & Smith, 1942*a*, *b*). In the References *all* names should be given in full but, where more than *two* collaborating authors are quoted in the text, the names are printed in full only at the first citation; after that the first name is followed by *et al.*

Illustrations

Text illustrations accompanying the papers should be drawn on smooth white Bristol board in Indian ink about twice the size of the finished block. Shading must be indicated by lines or dots. Graphs should be drawn boldly in Indian ink on white Bristol board or on white graph paper with pale-blue lines. All lettering of illustrations or graphs should be inserted clearly in pencil.

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